

HEAVY METAL COMPLEXATION WITH NATURALLY OCCURRING
ORGANIC LIGANDS IN WETLAND ECOSYSTEMS

by

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF THE UNIVERSITY
OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1981

ACKNOWLEDGMENTS

This dissertation would not have been possible without the assistance of many. I would like to express my sincerest appreciation to the chairman of my supervisory committee, Dr. P. L. Brezonik, for his assistance and guidance throughout this research project. Additionally, I would like to thank the other members of my committee—Drs. W. B. Arbuckle, G. R. Best, J. E. Singley, and J. J. Street—for their continued interest and guidance during this project.

The assistance of Jim Butner, Bill DeBusk, Pete Straub, and Bob Tighe in field efforts and sample analyses was invaluable. Special thanks go to Carl Miles, who always had time to discuss my half-baked ideas and who adroitly aided in many other phases of my research. Additional thanks go to all the other students and friends who provided moral and technical support.

Appreciation is extended to Jenny Carter for a highly professional job of typing this manuscript and to Randy Koper, Rick Cabrera, and Ruben Belen, Jr., for carefully drafting the figures.

I especially wish to thank my wife for her continued patience and support during my graduate studies and to my parents, who have always encouraged me to pursue further education.

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Abstract of Dissertation Presented to the Graduate Council of
the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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December 1981

Chairman: P. L. Brezonik

Major Department: Environmental Engineering Sciences

The mode, rate, and extent of heavy metal uptake by freshwater wetlands were investigated as well as heavy metal complexation by a variety of soluble organics. In one study, the levels of Cd, Cu, Mn, Ni, Pb, and Zn entering Basin Swamp from the sewage treatment plant in Jasper, Florida, were low (0.1–30 µg/L). Consequently, no conclusions could be made regarding heavy metal cycling in Basin Swamp. Another study involved adding Cd, Cu, Mn, and Zn to septage from Waldo, Florida, and pumping the wastewater through a 10 x 40-m corridor in a cypress swamp. Soluble concentrations of all four metals decreased within the corridor to levels close to those considered acceptable for discharge to surface water. Rates of immobilization were calculated to be a minimum of 7.2, 36, and 72, and 72 g metal/ha·day⁻¹ for Cd, Cu, Mn, and Zn, respectively. In addition, microcosms (model ecosystems that simulated a swamp) were constructed for a laboratory

investigation of factors that control metal immobilization rates. Of the factors—dissolved organic carbon, iron, calcium, sulfide, and pH—the latter two most greatly affected metal immobilization rates.

Heavy metal interactions with naturally occurring organics and model compounds were examined by complexometric titrations using five separate techniques. Three of the techniques used—anodic stripping voltammetry (ASV), ion-selective electrode, and fluorescence quenching—were previously published methods, and the other two methods—continuous flow ultrafiltration and competing ligand/differential spectroscopy—were developed for this study. Each procedure was used to determine the available metal binding ability of the organic solutions and the conditional stability constants of the metal-organic complexes. For each solution, agreement among the procedures was good except for the ASV method, which produced results lower than the others. Additionally, the ASV method yielded a conditional stability constant for copper with ethylenedinitrilotetraacetic acid that was five orders of magnitude lower than other published values. The low ASV results for copper with swamp-water organics was found to be caused by the reducible nature of organically complexed copper.

CHAPTER I INTRODUCTION

Increasing rates of pollution of natural waters by sewage and other anthropogenic sources has resulted in an increased environmental consciousness during the past quarter century and specifically has resulted in increasing concern about heavy metal cycling in the environment. The costs of achieving "zero discharge" (as required by 1985 by the Federal Water Pollution Control Act of 1972) by conventional waste-treatment methods have been found to be enormous. Furthermore, the stresses our burgeoning population has placed on our current freshwater resources have created considerable interest in utilizing land disposal of sewage effluents as a low-cost means of achieving tertiary treatment of wastewater and simultaneous recharge of freshwater aquifers.

In warm climates, such as the southeastern United States, flowing wetland ecosystems are a feasible alternative to in-plant tertiary wastewater treatment. However, the potential use of wetlands for sewage disposal is hampered by our lack of knowledge of heavy metal cycling in a wetland ecosystem. Specifically, the toxicity and mobility of heavy metals in wetlands remain in question. Although mechanisms of metal toxicity to the biota is currently an active area of research, a consensus among researchers is that toxicity is dependent upon the chemical form of heavy metals. Additionally, heavy metal transport in

an ecosystem varies among the species of metal. Thus, the necessity to speciate heavy metals is paramount in understanding heavy metal cycling.

One of the major obstacles to understanding heavy metal cycling in the environment is the lack of analytical methods that are capable of accurately speciating heavy metals. Consequently, the nature and extent of heavy metal-organic interactions in aquatic systems are not well understood.

The intent of the research proposed here was to investigate the mechanisms that govern the fate of heavy metals in a wetland ecosystem. Specifically, the objectives of this research were

1. to evaluate the precision and accuracy of analytical methods that are used to speciate heavy metals;
2. to quantify heavy metal interactions with organic matter from sewage, swamp water, and peat sediment; and
3. to determine the fate of heavy metals in cypress wetlands.

CHAPTER II LITERATURE REVIEW

A. Heavy Metal Content of Sewage

The concentration of heavy metals in treated sewage effluent is highly variable and extremely difficult to typify, primarily because of the variable nature of industrial loading to municipal sewage treatment plants. Oliver and Cosgrove (1974) monitored influent and effluent of a conventional secondary sewage treatment plant hourly during a 3-day period and reported metal concentrations varied as much as 100-fold. Presumably, metal disposal from industrial batch-type processes caused the observed fluctuations. Although the variable levels of heavy metals in sewage suggest that generalizations are at best tenuous, the concentrations of heavy metals in the secondary effluent of the Los Angeles sewage treatment plant are in the same general range as those reported by Klein et al. (1974) for wholly domestic sewage in New York and by Oliver and Cosgrove (1974) for a Canadian sewage treatment plant receiving domestic and industrial waste (Table II-1). To that extent, the values listed in Table II-1 can be considered "typical" of municipal sewage effluents.

The reported levels of metals in treated sewage are considerably higher than typical background levels of metals in freshwater (Table II-1). The largest differences between concentrations of metal in wastewater and freshwater appear for nickel and zinc. These metals

Table II-1. Concentrations ($\mu\text{g/L}$) of heavy metals in secondary effluent from domestic sources and in freshwater.

	Secondary Effluent Ontario ^a		Secondary Effluent Los Angeles ^b		Secondary Effluent New York ^c		Typical Freshwater ^d	
	Total	Dissolved (0.45 μ)	Total	Dissolved (0.2 μ)	Total			
Cd	1 ^e (<1-5) ^f	<1 ^e (<1-1)	8.59 (5) ^g	7.49	13 ⁱ		0.1	
Cr	60 (>20-680)	<10 (<10-20)	55 (85)	38	30		1	
Cu	80 (10-220)	70 (<10-180)	27 (55)	19	140		3	
Pb	15 (1-50)	6 (<1-14)	48 (85)	45	---		3	
Mn	40 (20-70)	30 (10-40)	25 (26)	24	---		8	
Ni	270 (<30-720)	220 (<30-580)	116 (165)	109	40		0.5	
Zn	560 (160-2410)	400 (60-1750)	103 (170)	90	160		15	

^aOliver and Cosgrove (1974).

^bChen et al. (1974).

^cKlein et al. (1974).

^dBowen (1979).

^eMean of 84 8-h composites.

^fRange of 84 8-h composites.

^gMean of two monthly composites.

^hMedian of 12 monthly composites.

ⁱMean of 270 daily samples.

(especially zinc) are more ubiquitous and less toxic than most of the other metals listed. Conversely, cadmium, which is the most toxic of the metals reported, was found at the lowest concentration. Furthermore, the metals in treated sewage are present primarily in the dissolved state (Table II-1), because heavy metals associated with particulates are effectively removed by conventional biological waste treatment (Chen et al. 1974).

Levels of heavy metals in domestic sewage effluent from three communities in Florida—Wildwood, Jasper, and a trailer park in Gainesville—were found to be 10–100 times lower than the values reported in Table II-1 for treated domestic sewage in New York and Los Angeles (Klein 1976; Boyt et al. 1977; Carricker 1977; Brezonik et al. 1981).

In these cases, reasons for the very low (i.e., near background) levels of metals are not readily apparent. Domestic sources of copper and zinc, for instance, include water pipes, foods, and other consumables (such as some shampoos, vitamins, and drugs), and, hence, one would expect to find these metals in domestic sewage.

Perhaps the lower levels of metals in sewage from the non-industrialized areas in Florida reflect the atmospheric concentrations of metals. Williams et al. (1974) reported atmospheric levels of heavy metals to be up to 100 times higher in industrial areas than in remote ones; hence, near New York and Los Angeles, higher levels of metals are expected in surface runoff, which often enters the sewage treatment facility.

Nonetheless, the presence of heavy metals in sewage effluent is potentially hazardous, and even in low concentrations, long-term discharge can be detrimental to a receiving ecosystem.

B. Fate of Metals in the Wetlands Environment

Heavy metals applied to wetland ecosystems may experience three pathways of transport and transformation: (1) uptake by plants; (2) movement in water to groundwater or surface water; and (3) immobilization in the soil matrix. The fate of heavy metals in freshwater cypress domes (ponds) was investigated by Klein (1976) and Carricker (1977), but the concentrations of heavy metals in the waste effluent (which was wholly from domestic sources) were too low to determine the ultimate fate of the metals. Similarly, Boyt et al. (1977) reported low concentrations of zinc, copper, and lead in the effluent from the Wildwood, Florida, sewage treatment plant and in the swamp receiving the waste. However, it should be noted that heavy-metals issues were a minor aspect of the study, and samples were collected on only one occasion at a few stations. In another study, Mudroch and Capobianco (1979) investigated a marsh that has received treated domestic sewage since 1919. Concentrations of metals in the surface water and sediment cores in the marsh were low and variable, and no trends were observed.

Natural (background) levels and cycling of metals in undisturbed swamp and marsh areas within Okefenokee Swamp were investigated by Casagrande and Erchull (1977). Concentrations of metals were reported for surface water, peat sediment, and 11 species of vegetation, and these results are summarized in Table II-2. Many of the reported values are less than the limit of detection since analysis employed the flame mode of atomic absorption spectroscopy, and hence the utility of much of the data is limited. In another study (Casagrande and Erchull 1976), metals in peat sediments (from the same locations described

Table II-2. Distribution of metals in Minnie's Lake Swamp and Chesser Prairie Marsh^a.

	Cr	Cu	Hg	Mn	Ni	Pb	Zn
<u>Minnie's Lake Swamp</u>							
Surface water (mg/L)	<0.04	<0.02	<0.0001	0.01	<0.06	<0.06	0.17
Peat (0-6 cm) (mg/kg dry wt.)	<16	12.5	0.73	7	6	18	14
Vegetation (mg/kg dry wt.)							
Mean of 14 samples	<4	1	0.26	196	6	<6	98
Minimum	<4	<1	0.05	7	<6	<6	8
Maximum	<4	4	0.98	701	10	<6	213
<u>Chesser Prairie Marsh</u>							
Surface water (mg/L)	<0.04	<0.02	0.0001	0.01	<0.06	<0.06	0.10
Peat (0-6 cm) (mg/kg dry wt.)	12	18	0.34	6	3	22	32.5
Vegetation (mg/kg dry wt.)							
Mean of 14 samples	--	4	0.93	46	8	8	177
Minimum	<4	<1	0.20	13	<6	<6	24
Maximum	7	8	2.04	131	16	18	366

^aCasagrande and Erchull (1977).

above) were examined in detail. The authors concluded that the metals were associated with the organic fraction of the peat sediments. However, metal concentrations did not correlate with depth of sediment, and hence, if the influx of metals to the swamp (and therefore the sediments) has increased due to cultural changes, the metals in the sediment were redistributed due to (1) the low pH of the sediment (pH 4), and (2) periodic oxidation of peat during droughts and fires (Casagrande and Erchull 1976).

Heavy metal cycling in a swamp containing naturally high levels of copper was reported by Dykeman and de Sousa (1966). Copper in the Tantramar Swamp, located in New Brunswick, Canada, originates from a mineral deposit in the bedrock beneath a 60-m layer of glacial drift and is circulated by percolating groundwater. The organic peat sediments contain up to 7% copper, yet the swamp supports a luxuriant growth of larch, black spruce, and ground cover species typical of other wetland areas in the region. The authors concluded that the reason for the apparent lack of copper toxicity to the swamp flora is due to the strong complexation of copper with the organic peat soil.

Although conclusive research about the fate of heavy metals in wetlands is sparse, several researchers have investigated heavy metal cycling in systems similar to freshwater wetlands. Banus et al. (1975) examined the fate of lead, zinc, and cadmium added to a salt marsh ecosystem and reported that most of the lead and cadmium added to the marsh was retained by the surficial sediments, while a small portion was taken up by the marsh grass. The salt marsh acted as a partial sink for zinc, with substantial export of zinc to deeper water.

Jennett and Linnemann (1977) reported excellent retention of zinc and

lead applied to Missouri soil under laboratory conditions. The absorption capacity of the soil approached 100% of the cation exchange capacity (CEC), illustrating the utility of the soil for land disposal of waste from milling and mining operations.

Waterhyacinths, which are frequently found near the nutrient-rich effluents of sewage treatment plants, have been shown to accumulate heavy metals (Dinges 1978). The leaves and stems of the hyacinth culture that received treated sewage contained high levels of Cr, Cu, Fe, Hg, Mn, Ni, and Zn, whereas Ag, Cd, and Pb contents were below the reported detection limits.

Empirical evidence suggests that sediment-bound heavy metals are associated with several geochemical phases, the principal ones being hydrous metal oxides, clays, organics, carbonates, and sulfides (Jenne 1968; Khalid et al. 1977). Since the bioavailability and recycling of heavy metals depend on the chemical phase with which a metal is associated in sediments (Jenne and Luoma 1975), the quantitative distribution of metals among the various phases is of considerable importance.

To determine the distributions, several researchers have proposed schemes by which metals are selectively extracted from natural sediments to determine metal partitioning (Presley et al. 1972; Gupta and Chen 1975; Engler et al. 1977; Tessier et al. 1979). However, these extraction schemes are laden with difficulties, such as low recovery of metal and non-specific extraction. Moreover, there is a considerable lack of information on desorption/adsorption kinetics (Luoma and Jenne 1976; Guy et al. 1978). At present, sediment extraction schemes cannot adequately assess heavy metal partitioning in the solid phase.

Even though the chemical form of metals in sediments is not amenable to accurate analysis, several reports have indicated that heavy metals can be immobilized by insoluble organic matter similar to the organic peat found in wetlands. Chaney and Hundemann (1979) added industrial effluent containing 100 ppm cadmium to 60-cm long columns containing peat moss, and they found 2 ppb cadmium in the effluent. Analysis of the column following the passage of 14 L of effluent showed that most of the cadmium was present in the upper few centimeters of the column, indicating that organic peat moss was efficient in removing cadmium. In a similar study by Coupal and Lalancette (1976), peat moss was used in pilot plant scale (20,000 gpd) to remove Hg, Pb, Sb, Cu, Ni, Zn, and Cr from a variety of industrial wastes. These workers found the organic peat to be effective in reducing the levels of all metals to concentrations ranging from 0.02 to 0.2 mg/L.

In light of the previous discussion, a priori one would expect the insoluble peat, which usually composes a major portion of sediments in wetlands, to be at least partially responsible for immobilizing soluble heavy metals. A variety of factors, such as the concentration of soluble organic matter, the pH of the water, and sulfide levels, would be expected to affect the rate and degree of metal immobilization by peat sediments. However, the influence of these and other environmental parameters on the fate of heavy metals has not been addressed satisfactorily to date.

C. Organic Composition of Sewage and Swamp Water

Both sewage effluent and humic-colored surface water (such as those in cypress swamps) are rich in organic matter that may alter the rate at which metals are removed from solution. For instance, Davis and Leckie (1978) proposed three mechanisms by which soluble organic matter can affect adsorption of metals to solids (either suspended particulates or sediments):

- (1) organic ligands not adsorbed to solid surfaces can decrease adsorption of metals due to complexation of the metals;
- (2) organic ligands adsorbed to surfaces, but not capable of ligand-bridging with metals, can decrease adsorption by decreasing the availability of surface sites; and
- (3) complex-forming organic ligands strongly adsorbed to surfaces can function as effective ligand bridges and increase adsorption.

In addition, soluble organic matter has been reported to enhance uptake of heavy metals by duckweed (Carriker 1977), stimulate algal growth in seawater (Barber 1973; Sunda and Guillard 1976; Anderson and Morel 1978), and reduce toxicity of heavy metals to fish (Stiff 1971b; Zitko et al. 1973). At this time, no consensus exists regarding the mechanism by which organic matter ameliorates the toxic effects of heavy metals to the biota.

The literature concerning the importance of complexation of heavy metals by organic matter is vast and reviewed thoroughly elsewhere (Singer 1973; Leckie and James 1974; Jenne and Luoma 1975; Reuter and Perdue 1977). However, because the extent of heavy metal complexation

is related to the chemical nature of organic matter (i.e., functionality, molecular size and shape), the reported organic composition of sewage and cypress swamps will be discussed in the following section.

1. Sewage Organics

In the case of a cypress swamp receiving heavy metal-containing waste, the metals likely would be complexed initially with the organic matter present in the wastewater. The most detailed characterization of organic matter in secondary sewage was reported by Manka et al. (1974). They investigated three Israeli treatment facilities, which included trickling filter, stabilization pond, and extended aeration-activated sludge treatments. The organic composition was similar among the different facilities, with average values (as percent of COD) as follows: humics, 43%; proteins, 22%; anionic detergents, 15%; ether extractables, 14%; carbohydrates, 6%; and tannins, 1.5%.

The composition of total nitrogen in the influents of two municipal sewage treatment plants in Wisconsin was studied by Hanson and Lee (1971). Average total hydrolyzable amino acid levels were similar for the two influents (2.1 and 2.7 mg N/L). Ammonia and urea represented most of the remainder of total nitrogen, although the results were variable and a significant portion of organic nitrogen remained unidentified. Keller et al. (1978) examined the behavior of soluble organic nitrogen from a secondary effluent on a column of Sephadex G-15 and found that the organic nitrogen was distributed over the entire molecular weight range of the column (i.e., <165->1800 daltons). Their

results imply that the organic nitrogen in secondary sewage effluents is heterogeneous, and not of a single molecular size.

2. Swamp Organic Matter

The colored organic matter typically found in surface water of swamps and marshes has been classified collectively as humic substances. Additionally, the terms organic color, yellow organic acids, gelbstoff, humates, fulvic acids, and humic acids have been used in the literature to identify this class of compounds.

Humic substances are subdivided into three fractions: (1) humic acid, which is soluble in alkaline solution (0.1–0.5 M NaOH) but is precipitated by acidification (pH 1); (2) fulvic acid, which is the humic fraction that remains soluble in both alkaline and acidic solutions; and (3) humin, the fraction that cannot be extracted by acid or base (Kononova 1966). Although this fractionation scheme is arbitrary and the fractions are molecularly heterogeneous, Schnitzer and Khan (1972) have reported some properties that distinguish fulvic and humic acids. Fulvic acid has lower color, carbon content, and molecular weight and higher CEC, oxygen content, and density of functional groups than does humic acid. Therefore, due to the more soluble nature of fulvic acid, the humic substances in natural water are predominantly the fulvic acid fraction.

The structure and function of humic substances have been the topics of much research, and because several excellent reviews are available (Faust and Hunter 1971; Schnitzer and Khan 1972; Povoledo and Golterman 1973; Gjessing 1976; Carriker 1977; Miles 1979; Hartenstein

1981), only a brief discussion of the chemical nature and dynamic role of humic substances in natural waters is presented here.

No one has satisfactorily characterized humic substances, and no consensus exists regarding the structure of these compounds; however, several hypothetical structures have been proposed that account for their elemental composition, degradation products, acidic properties, and complexation capacities (Christman and Ghassemi 1966; Schnitzer 1971; Stevenson and Ardakani 1972; Gamble and Schnitzer 1973). The structures consist mainly of complex hydrophilic polyelectrolytic polymers of benzene rings that are variously substituted with phenolic, carboxylic, or methoxy groups, together with large amounts of aliphatic carboxylic acids. Two hypothetical structures of humic materials—one that is loosely held together by hydrogen bonds and another that is more rigidly bonded—are illustrated in Figure II-1. The elemental composition varies among sources and analytical techniques, but typical ranges are: C, 45–63%; O, 30–35%; H, 3–6%; N, 0.5–5% (Gjessing 1976; Schnitzer and Khan 1972). Molecular weights of these polymers have been reported to range from a few hundred to a few hundred thousand, although recently a consensus has been growing that aquatic humic substances are approximately 1000 daltons (Wilson and Weber 1977; Reuter and Perdue 1981; Schnitzer 1981). These workers based their conclusions on colligative property techniques such as vapor phase osmometry and cryoscopy. Interestingly, Shapiro (1964) concluded similarly that aquatic humic material was of low molecular weight (using cryoscopy), but because several others reported much higher molecular weights for humics (Black and Christman 1963; Gjessing and Lee 1967; Ghassemi and Christman 1968; Gjessing 1971), Shapiro's results were disbelieved

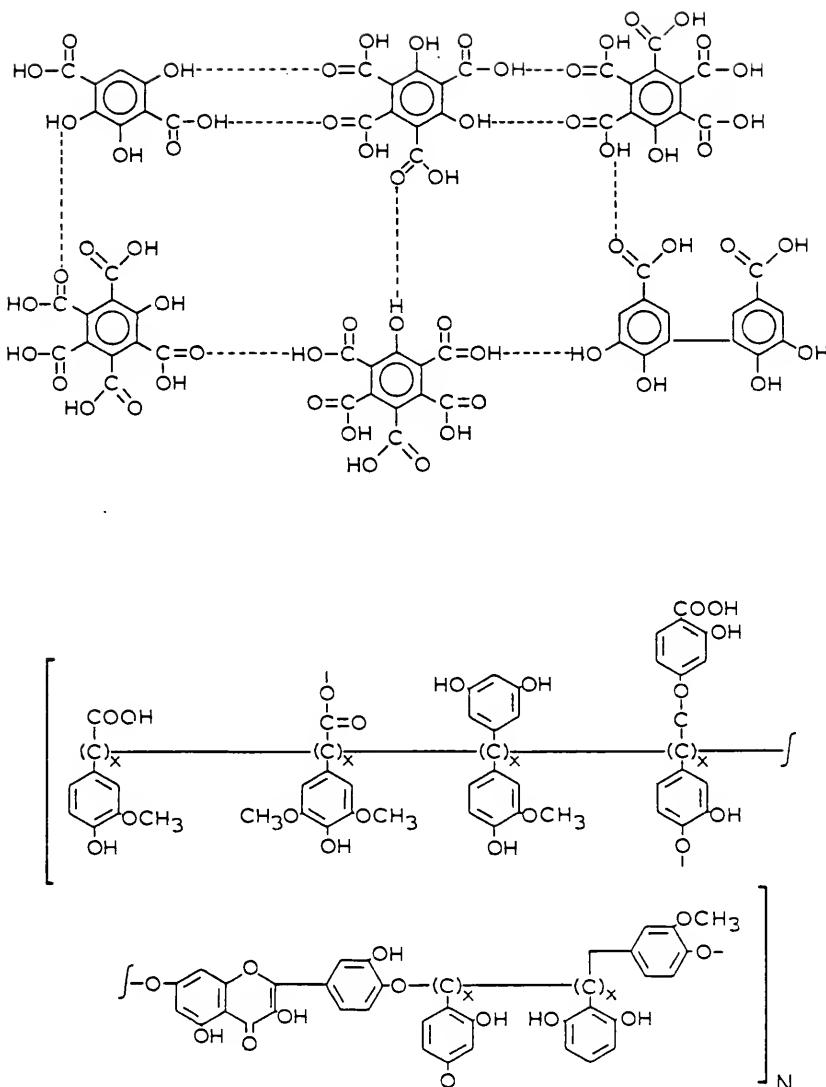


Figure II-1. Proposed structure of humic substances from Schnitzer (1971) (top) and Christman and Ghassemi (1966) (bottom).

until recently. The high molecular weights were determined using gel chromatography, ultrafiltration, and dialysis techniques, which can cause aberrant results due to adsorption and charge repulsion of the organic solute with the membranes and gel beads (Tuschall and Brezonik 1980; Truitt and Weber 1981b).

Heavy metal complexation with humic materials has been reported by many researchers, and several reviews are available (Singer 1973; Rubin 1974; Reuter and Perdue 1977). Of the metals commonly found or expected in a wetland environment, copper and iron typically form the strongest complexes with humics; manganese and zinc form the weakest complexes; and lead, nickel, and cadmium form complexes of intermediate stability. The conditional stability constants, β' (defined in Section II-D), vary greatly due to both the variability of the methods used to determine β' and the variability of the conditions of analysis, especially pH. Nevertheless, the overall implications of past research are that humic materials can complex heavy metals to an appreciable extent, thus altering the toxicity, solubility, and ultimately the fate of heavy metals in the environment.

D. Methods for Quantifying Metal-Organic Matter Interactions

The most commonly employed parameters for quantifying the extent and stability of complexation between heavy metals and naturally occurring organic matter are available metal binding ability of the organic matter, also called complexing capacity (C_L), and the conditional stability constant (β'). Available metal binding ability is defined as the quantity of a metal ion titrant that is complexed by the ligands in

a water sample and is usually expressed in units of moles/L (Allen et al. 1970; Chau 1973; Hanck and Dillard 1977b). Typically, C_L is determined by titrating a sample with the metal of interest and, following each addition of titrant, measuring the concentration of complexed metal (e.g., ion-exchange technique [Crosser and Allen 1976]), the concentration of uncomplexed or weakly complexed metal (e.g., ion-selective electrode [Buffle et al. 1977], anodic stripping voltammetry [ASV] [Shuman and Woodward 1973, 1977], equilibrium dialysis [Zunino and Martin 1977; Truitt and Weber 1981b]), or the concentration of uncomplexed ligand (e.g., fluorescence quenching [Saar and Weber 1980]). The point at which the ligand can no longer complex additional metal ion is the available metal binding ability (or complexation capacity) of the solution, which can be determined graphically.

In describing the quantitative stability of a complex for the equilibrium condition



the thermodynamic stability constant, β , is defined:

$$\beta = \frac{(M_aL_b)}{(M)^a(L)^b}, \quad (2.2)$$

where (M) is the activity of free metal ion, (L) is the activity of the reactive species of ligand, and (M_aL_b) is the activity of the complex. The value of β is a true constant, although the value alone says nothing about the distribution of M and L under existing solution conditions such as pH or concentrations of other competing species, nor does β reveal anything about the distribution of M and L in reference

to their total concentrations, since the metal and ligand can exist in forms other than M^{+b} , L^{-a} , and M_aL_b (other forms include MOH , MCO_3 , HL , H_2L , etc.). Furthermore, β is extremely difficult (or impossible) to determine accurately for environmental samples, which contain a heterogeneous mixture of ligands, since the various side reactions and their attendant constants are seldom known and are exceedingly difficult to obtain. For these reasons, another parameter, the conditional stability constant, β' , was proposed by Ringbom (1963) for the equilibrium condition $aM + bL = M_aL_b$. The conditional stability constant, β' , is defined by Ringbom (1963) as follows:

$$\beta' = \frac{(M_aL_b)}{(M')^a(L')^b} \quad (2.3)$$

where (M') is the concentration of metal not complexed with L , and (L') is the concentration of L not complexed with M . By definition, β' is not a constant, but instead is a function of the solution conditions and varies with concentrations of competing species such as hydrogen ion, calcium, and carbonate, and also with metal and ligand concentrations (Ringbom 1963; Mantoura and Riley 1975; Bresnahan et al. 1978; Buffle 1980; Gamble et al. 1980). Nevertheless, β' is a useful parameter for predicting the relative distribution of metal for specific environmental conditions.

Numerous methods exist for determining the available metal binding capacity (C_L) of naturally occurring organic matter and conditional stability constants (β') for the resulting complexes. In the following section, the most commonly used procedures are described briefly and the utility of each method for naturally occurring organic matter is

discussed. These methods can be grouped into major classes as follows.

1. Physical Separation Techniques

The gel permeation chromatography method described by Mantoura and Riley (1975) involves a dynamic equilibrium between organic matter that is injected onto a chromatographic column of porous gel beads and metal ions in the elution buffer. The complexes formed between the macromolecular organic matter and the metal ions are excluded from the gel beads and travel down the column more rapidly than the free metal ions, which permeate the gel. A plot of metal concentration versus volume of eluant (Figure II-2) shows a peak due to complexed metal (peak A) at the same volume in which the organic ligand eluted, and a negative peak (metal deficiency) at the volume corresponding to elution of low molecular weight solutes (peak B). The areas of the two peaks determine the amount of metal complexed, and knowledge of the initial ligand concentration and the background metal concentration permits calculation of conditional stability constants. For a 1:1 complex (i.e., ML), β' is calculated as follows:

$$\beta' = \frac{(ML)}{(M')(L')} = \frac{(M_{\text{bound}})}{(M_f)(L_t - M_{\text{bound}})} \quad (2.4)$$

where $ML = M_{\text{bound}}$ = area of peak A (Figure II-2), M_f is the uncomplexed metal concentration (i.e., metal concentration in eluant), and L_t is the total ligand concentration. Multiple chromatographic runs at varying metal concentrations can yield information about different metal binding sites on one ligand or sequential complexation by a mixture of ligands.

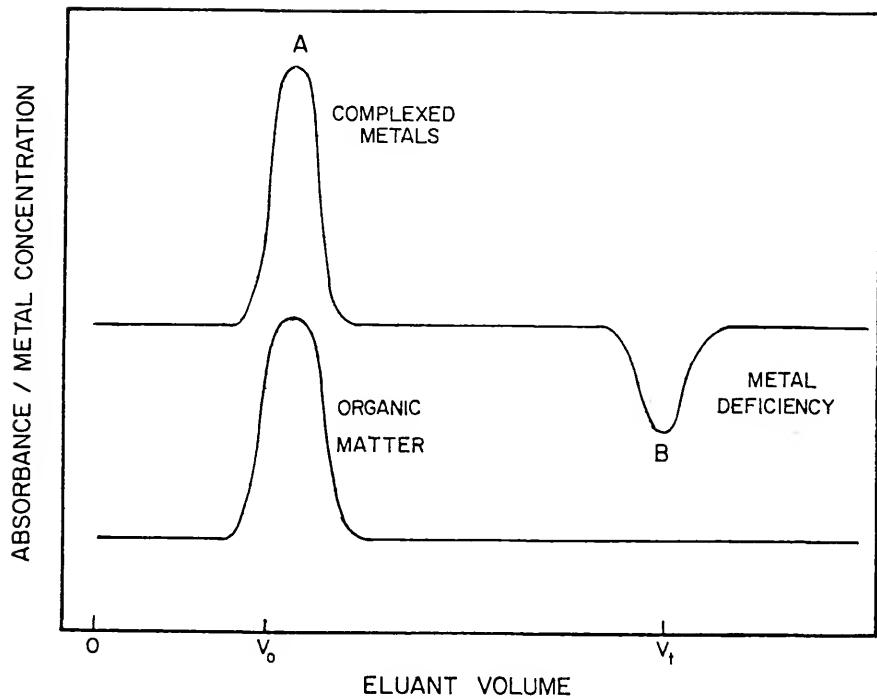


Figure II-2. Theoretical gel filtration chromatogram for the interaction of organic matter with metal ions.

Unfortunately, this method is limited to only those organic ligands that are excluded by the gel beads. The minimum exclusion limit for Sephadex brand gels (G-10) is 700 daltons, and for Biogel brand gels (P-2) is 1800 daltons. Solute-gel charge interactions are significant, and adsorption of organic matter to the gel (especially humic substances at low ionic strength and pH) often necessitates conditions of analysis that are not typical of the natural system (i.e., 1 M ionic strength and pH 8.0) (Klein 1976; Miles 1979). However, this method is applicable to a variety of metals (with some exceptions such as Fe). Mantoura and Riley (1975) and Mantoura et al. (1978) reported conditional stability constants with Cd, Co, Cu, Hg, Mn, Ni, and Zn using the gel filtration chromatographic technique.

Membrane separation is another method available to measure C_L and β' of metal-organic complexes. The two types of membranes most commonly used for studying metal-organic matter interactions are dialysis tubing and ultrafiltration membranes. For the dialysis method, a sample is placed inside semipermeable cellulose-based tubing, which subsequently is suspended in a large reservoir of buffered water. Small ions and compounds diffuse through the membrane until equilibrium is reached, whereas large organic molecules (and heavy metals complexed to them) are prevented from passing through the tubing. Analysis of the two solutions for total metal concentrations at equilibrium indicates the quantity of metal bound to the retained organic matter. The initial concentrations of metal and organic matter can be varied, and a complete binding analysis can be performed. This method has been used to study protein-metal interactions (Klotz et al. 1946; Gurd and Goodman 1952; Malmstrom 1953; Osterberger 1973) and metal binding by humic

substances (Benes et al. 1976; Guy and Chakrabarti 1976; Zunino and Martin 1977; Truitt and Weber 1981b). Unfortunately, the dialysis procedure suffers from several important problems. Metals and organic matter may adsorb to the membrane; the membranes are permeable to some environmentally important organic compounds; and the necessity to repeat the experiment at several different metal or ligand concentrations makes the procedure tedious and time-consuming (Ramamoorthy and Kushner 1975; Zunino and Martin 1977; Truitt and Weber 1981b). On the other hand, the dialysis procedure works well at pH and ligand concentrations typically found in natural waters, and most metals can be speciated by dialysis.

The other common membrane-separation technique is ultrafiltration. In this procedure, a membrane composed of a non-cellulosic polymer lies on the bottom of a pressurized, stirred cell that contains a solution of the metal and ligand of interest. Pressure forces water containing small ions and compounds to pass through the membrane, whereas large compounds are retained. Analysis of the retained and filtered fractions indicates the extent of metal binding with high molecular-weight organic matter. Ultrafiltration is subject to the same advantages and disadvantages as the dialysis method, although the former method has the added advantage of controlled and variable rate of separation because the system is pressurized.

Most of the ultrafiltration studies reported to date were designed to speciate ambient levels of metals, and therefore filtrations were performed at a single metal and ligand concentration (Gjessing 1970; Schindler et al. 1972; Benes and Steinnes 1974; Guy and Chakrabarti 1976; Giesy and Briese 1979; Hoffman et al. 1981). Titrations of

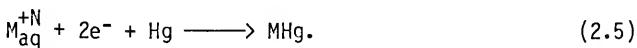
organic matter with heavy metals have been avoided, apparently due to the tedium involved in performing individual experiments for each addition of metal. Ultrafiltration also has been used simply to fractionate organic matter into different size ranges, and other metal-speciation techniques were used to investigate interactions of heavy metals with the fractionated organic matter (Allen 1976; Smith 1976; Hoffman et al. 1981).

2. Potentiometric Titrations

Anodic stripping voltammetry (ASV) has been used frequently in studying metal-organic matter interactions in natural waters, and the method of complexometric titration has been developed to determine the available metal binding capacity (C_L) and conditional stability constant (β') (Matson 1968; Shuman and Woodward 1973, 1977; Chau and Lum-Shue-Chan 1974). The method involves titrating water samples that contain excess ligand with a heavy metal and analyzing the "uncomplexed" metal by ASV. The endpoint of such a titration is interpreted as a measure of the complexing capacity (C_L) of a water sample. The original procedure was adapted by Shuman and Woodward (1973, 1977) to include the direct determination of a conditional stability constant (β') for metal-ligand complexes that are not reduced under the conditions of metal ion analysis.

The ASV analysis consists of two steps: an initial plating step and a subsequent stripping step. In the former step, a potential more negative than the reduction potential of the analyte metal ion is applied to the solution, thus reducing the metal to an uncharged state.

The elemental metal plates onto the electrode, which is typically mercury, and thus the analyte is concentrated during the plating step:



Subsequently, the electrode potential is increased linearly with time in the stripping step, causing the metals that have been concentrated on the electrode to be oxidized and to flow back into solution. Oxidation occurs at a characteristic potential (E_p) for each metal; concurrent with oxidation is a release of electrons. The electron flow is measured as current (i) during the scanning step. A typical ASV scan of a 10 μM copper solution is illustrated in Figure II-3. If all other variables such as stirring rate, deposition time and voltage, scan rate, and electrode surface area remain constant, the measured peak current (i_p) is directly proportional to the concentration of reducible metal originally in solution. Hence, a plot of peak current (i_p) versus metal added produces a titration curve from which the values of C_L and β' can be obtained. The theoretical considerations of calculating C_L and β' are discussed further in Section III-D.

The ASV titration method is a simple technique that can be used at metal, ligand, and pH levels found in natural waters. However, the authors who developed the method stated the following underlying assumptions: (1) i_p is proportional to free and labile metal; (2) the ML complex is non-reducible; (3) the ML complex does not kinetically dissociate appreciably during the plating step (Shuman and Woodward 1977). Although Shuman and Woodward (1977) stated that these assumptions appear reasonable for natural water samples, the validity of the assumptions has not been demonstrated clearly. There is reason to believe that the dissociation or direct reduction of metal-ligand

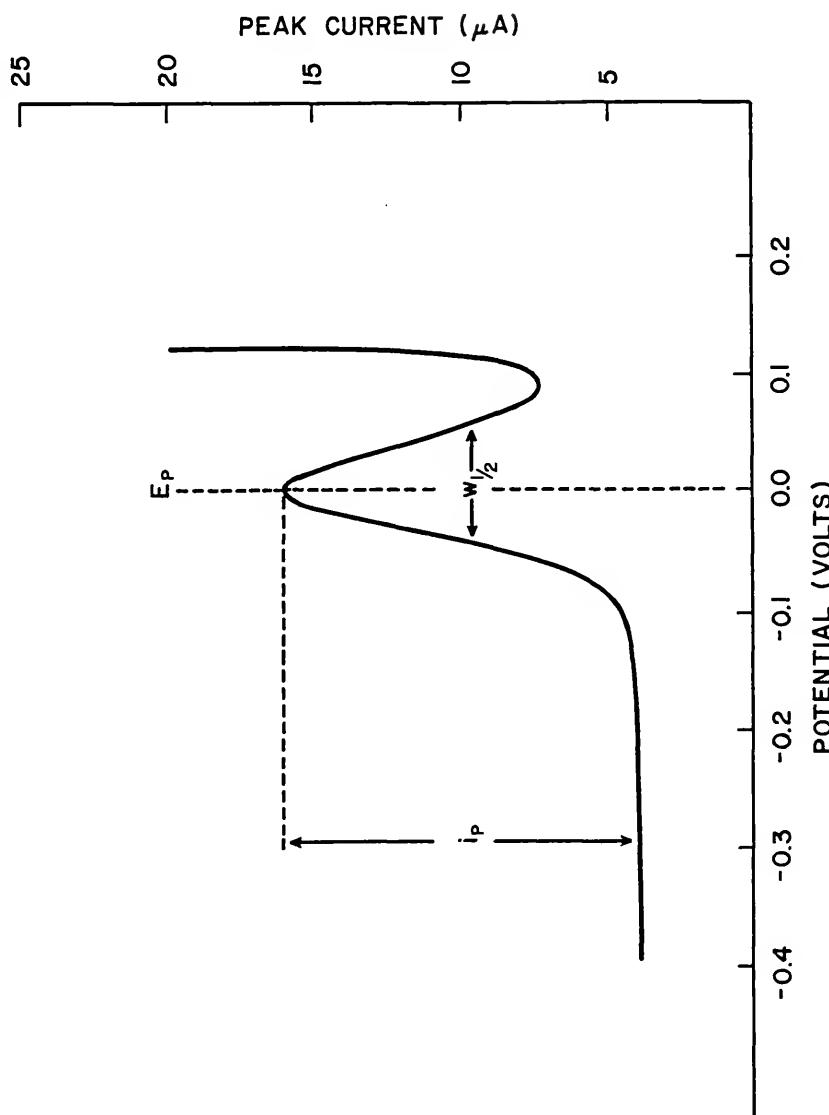


Figure II-3. Typical ASV scan of a sample containing 10 μ M copper.

complexes can cause low results. For example, Hanck and Dillard (1977b) reported up to 34% relative error in determining the complexation capacities of ethylenedinitrilotetraacetic acid (EDTA) solutions using indium as the titrant, and they assumed that complex dissociation caused the low results. Nonetheless, copper is commonly used to titrate natural water samples (Chau and Lum-Shue-Chan 1974; O'Shea and Mancy 1976; Shuman and Woodward 1977; Sugai and Healy 1978; Tuschall and Brezonik 1980) because it forms more stable complexes with most organic ligands than do most other environmentally significant metals such as zinc and cadmium (Irving and Williams 1948). However, the extent of complex dissociation or reduction remains a potential drawback of using copper as titrant.

Ion-selective electrodes have been used widely in characterizing interactions of heavy metals with naturally occurring organic matter (Stiff 1971a, 1971b; Gardiner 1974; Buffle et al. 1977; Bresnahan et al. 1978; Swallow et al. 1978; Giesy and Briese 1979; McKnight and Morel 1979; Saar and Weber 1980). Presently, the only heavy metals for which ion-selective electrodes are commercially available are Cu^{+2} , Cd^{+2} , Pb^{+2} , Hg^{+2} , and Ag^{+1} . These electrodes respond to the aquated ion only and not to any complex forms of metal.

The electrode response is described by the Nernst equation:

$$E = E^\circ + \frac{2.303 RT}{nF} \log a_M^{+n} \quad (2.6)$$

where E° is the standard electrode potential for the metal being measured, a_M^{+n} is the activity of metal ion, and $2.303 RT/nF = 29.6 \text{ mV}$ at 25°C for a divalent ion ($n = 2$) and 59.2 mV for a monovalent ion ($n = 1$) (Ross 1969). Hence, for each 10-fold change in activity,

the electrode response (E) should change by 29.6 mV for Cu^{+2} , Hg^{+2} , Cd^{+2} , Pb^{+2} and 59.2 mV for Ag^{+1} .

Ion-selective electrodes allow direct measurement of heavy metal ion activity at pH and ionic strength levels found in most natural waters. However, a minimum total heavy-metal concentration is required to obtain accurate potentials for heavy-metal ion activity; thus typical environmental levels of metals cannot be detected using ion-selective electrodes. For instance, the limit of detection of the Cu^{+2} -ISE is about 10^{-7} M Cu^{+2} (6.5 $\mu\text{g Cu}^{+2}/\text{L}$) for a solution containing aquated copper (Cu^{+2}) as the only form of copper. However, for a solution with complexed copper in addition to aquated copper (Cu^{+2}), the ISE will measure levels of Cu as low as 10^{-10} M if the total concentration of copper is 10^{-7} M or more (McKnight and Morel 1979). For this reason, ion-selective electrodes are best suited for laboratory studies involving sample titrations with heavy metals. Disadvantages of using ion-selective electrodes include lengthy calibration procedures that need to be performed frequently due to drift, and broad titration curves that make C_L difficult to determine accurately.

3. Ion Exchange

Shubert (1948) developed the ion-exchange technique to determine C_L and β' for a metal-organic matter system, and, subsequently, the method of calculating β' has been revised and adapted by Zunino et al. (1972), Crosser and Allen (1976), and Stevenson and Ardakani (1972). Basically, the ion-exchange procedure is performed by adding a strong cation exchange resin (e.g., Dowex 50) to a series of vessels

containing the ligand of interest at several concentrations of metal. Flasks are mixed for a specified time (e.g., 24 h), and the supernatants are analyzed for total soluble metal by atomic absorption spectroscopy. The amount of metal taken up by the resin is the difference between the amount added initially and the amount remaining in solution at equilibrium. The soluble metal concentration is related to the extent of metal complexation: that is, complexed metal that does not adsorb to the resin will increase the concentration of soluble metal compared to that in the absence of ligand. Theoretical plots of metal bound by the resin versus total soluble metal for three possible cases are illustrated in Figure II-4. The curves vary according to the stabilities of the metal-ligand complexes formed, the ligand concentration, and the number of ligands present.

The principal advantage of the procedure is that any metal that can be analyzed by atomic absorption spectroscopy can be used, unlike methods using ASV or ion-selective electrodes, in which only a few metals can be analyzed. However, the ion-exchange procedure does not work well with weak ligands because partitioning of metal between solution and resin strongly favors the resin, and hence only a small proportion of metal remains in solution. Thus for ligands that form weak complexes, a weak cation exchange resin such as manganese dioxide is preferable, although preparation of a reproducible suspension of MnO₂ is difficult and highly dependent on technique (van den Berg and Kramer 1979). Another serious problem with the ion-exchange technique is adsorption of ligand to the surface of the resin, thus reducing both the extent of metal exchange between solution and resin and the soluble ligand concentration.

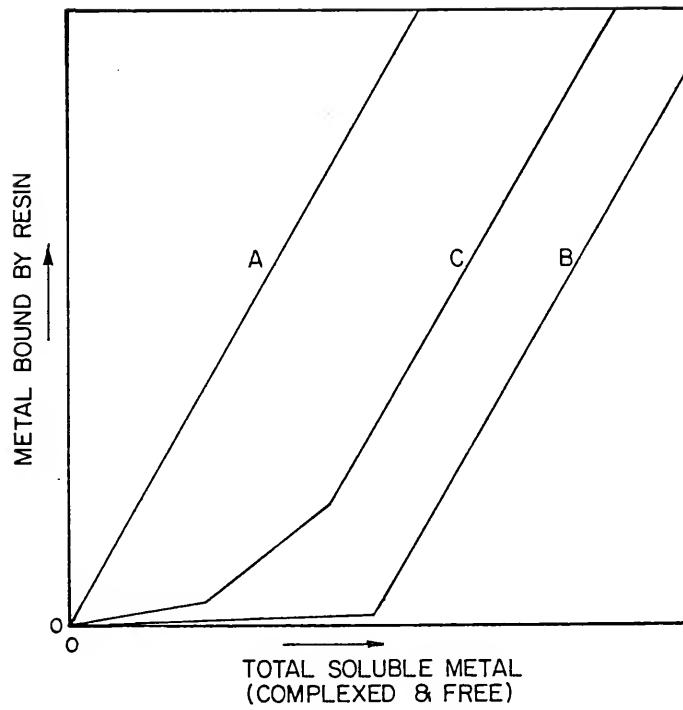


Figure II-4. Theoretical curves for ion-exchange equilibrium method (A: no ligand present; B: strong ligand; C: mixture of two ligands, both weaker than ligand in B).

4. Spectrophotometric Methods

The fluorescent properties of naturally occurring organic matter have been used to quantify metal-organic interactions (Levesque 1972; Saar and Weber 1980). Fluorescence of organic matter is diminished (quenched) by binding with paramagnetic metal ions such as Cu^{+2} , Co^{+2} , and Ni^{+2} (i.e., ions with one or more unpaired electrons), and thus the intensity of fluorescence can be used as a measure of ligand not bound to paramagnetic metal ions. Metal binding ability is determined simply by titrating organic matter with metal and recording fluorescence after each addition. Although the technique is simple to perform and sensitive to low levels of ligand, only fluorescent ligands and paramagnetic metal ions can be analyzed by this method.

Job (1928) introduced the method of continuous variation for determining C_L and β' . In this method, differential spectroscopy is used to measure the absorbance of solutions containing various levels of metal and ligand. One condition necessary to perform this analysis is that the ML complex absorbs light at a characteristic wavelength, and, ideally, the other metal and ligand species do not absorb appreciably at the analytical wavelength. Background absorbance is usually subtracted optically by placing the sample without added metal in the reference path, and the sample with added metal in the sample path (i.e., differential spectroscopy).

By mixing V volumes of ligand (concentration L_0) with $1-V$ volumes of metal (concentration $M_0 = L_0/r$), where V varies from 0 to 1, the concentration of the ML_x complex (i.e., Δ absorbance) will be maximal when

$$\beta' M_0 r^{x-1} [(x+r)V_{max}-x]^{x+1} = (r-1)^x [x-(x+1)V_{max}]. \quad (2.7)$$

Thus a plot of Δ absorbance versus the fractional volumes of metal and ligand (Figure II-5) is used to determine V_{\max} , and ultimately β' using equation 7. However, the method of continuous variation is usually not sensitive to low levels of metal and ligand, and it suffers from the underlying assumption that only one complex is formed throughout the titration.

The concept of differential spectroscopy has been applied further to the determination of β' of pure compounds by monitoring the concentration of metal complexed with a competing ligand (Rossotti and Rossotti 1961). In this procedure, a competing ligand (A) that has a known stability constant with the titrant metal is added to a solution of the metal (M) and ligand (L) of interest, and the following equilibria are established:



By measuring the concentration of MA (using differential spectroscopy) and knowing the analytical concentrations of M, L, and A, the conditional stability constant of the ML complex can be calculated.

The competing-ligand method using differential spectroscopy has been applied to environmental samples by Anderegg et al. (1963) and Harris et al. (1979), who used EDTA as the competing ligand to determine the conditional stability constants for iron with several microbially produced siderophores. Campbell et al. (1977) reported a competing-ligand procedure to study binding of zinc by natural ligands in river water. The competing ligand was a zinc-specific ligand called zincon (2-carboxy-2'-hydroxy-5'-sulfoformazylbenzene), which forms a red complex with zinc. The concentration of the zinc-zincon complex,

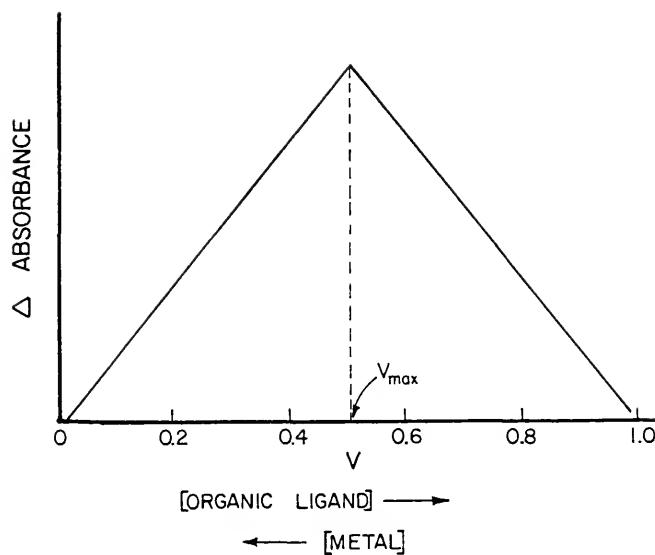


Figure III-5. Theoretical curve for method of continuous variations.

monitored in the presence of naturally occurring organic matter using differential spectroscopy, was used to determine the complexation capacity (C_L) of the river water samples.

Unfortunately, many of the strong absorption bands for metal-ligand complexes are in the 200–300-nm range (Martin 1974), which is the same region that natural organic matter absorbs strongly (Schnitzer and Khan 1972). Although the background absorbance can be subtracted optically, the accuracy of measuring a small MA peak in the presence of a strong absorption band is diminished. Nevertheless, if an appropriate competing ligand is used, the method has the advantages of being simple to perform, sensitive to low levels of metal and ligands, and often applicable to more than one metal (Rossotti and Rossotti 1961).

5. Miscellaneous

In addition to the methods discussed in the preceding section, several other methods for quantifying metal-organic interactions have been proposed, although their use for natural water studies has been slight. These include a copper solubilization method (Kunkel and Manahan 1973), a potentiometric titration using a pH electrode (Stevenson 1977), and a Co(III) complexation technique (Hanck and Dillard 1977a). Apparently procedural limitations (such as low sensitivity, restricted sample pH, and excessive sample manipulation) have prevented these methods from receiving widespread use.

As the above review indicates, none of the currently available methods for quantifying metal-organic interactions is problem-free, and many suffer from serious limitations. The need to critically evaluate

the accuracy of existing methods and to develop new and better methods is apparent if metal-organic interactions in natural waters are to be characterized accurately.

E. Methods of Fractionating Organic Matter

Interactions between heavy metals and naturally occurring organic matter have been studied by numerous researchers, and typically these studies include determination of conditional stability constants for the metal-organic complex (Mantoura and Riley 1975; Guy and Chakrabarti 1976; Buffle et al. 1977; Bresnahan et al. 1978; Davis and Leckie 1978). Although several different methods have been used to determine stability constants for copper-organic complexes, one interesting trend emerged when Scatchard plots were used to calculate the constant: namely, that two sites of metal bonding were observed, one strong and one weak (Mantoura and Riley 1975; Guy and Chakrabarti 1976; Bresnahan et al. 1978; Giesy 1978).

A priori, one would expect the strong site to be very important in heavy metal partitioning. Fractionation of the organic matter prior to measuring metal binding ability may determine if the strong and weak binding sites exist as separate entities.

Rebhun and Manka (1971) developed a fractionation procedure that involved a series of chemical precipitations and solvent extractions to fractionate the organics in secondary sewage. Their scheme was used to isolate anionic surfactants, carbohydrates, tannins, proteins, fulvic acids, humic acids, hymatomelanic acids, and ether extractables. However, chemical precipitation and solvent extraction techniques

suffer from the problems of contamination and poor recoveries, and extreme care must be exercised to minimize these problems.

Sirotkina et al. (1974) fractionated dissolved organic matter from the Moscow River by using columns of ion-exchange celluloses followed by further separation on gel permeation bead columns. Their scheme, illustrated in Figure II-6, involved application of a sample to an anionic exchange column (DEAE cellulose), onto which the anionic organics (acidic fraction) were adsorbed and retained. The cationic and neutral organic matter in the elute was subsequently applied to a cation exchange column (CM cellulose), which retained the cationic organic matter. The adsorbed compounds were released from the columns by rinsing with 0.1 N NaOH (DEAE column) or 0.1 N HCl (CM column). The desorbed organic matter and the neutral fraction were separated further using a column of Sephadex gel. The authors claimed better than 90% recoveries of organic matter, which is superior to most reported recoveries for similar techniques using other ion exchange resins (i.e., divinyl benzene or styrene polymer supports). These resins typically adsorb organic matter irreversibly (Sirotkina et al. 1974; Leenheer 1981).

An approach similar to that described by Sirotkina et al. (1974) was used by Tuschall and Brezonik (1980) to isolate the proteinaceous fraction of organic matter from water. They applied an acidified sample to a strong cation exchange column (P cellulose), desorbed the retained organic matter with NaOH, and desalinated the isolated fraction with a Sephadex column. Recoveries of organic matter from the column were greater than 90%.

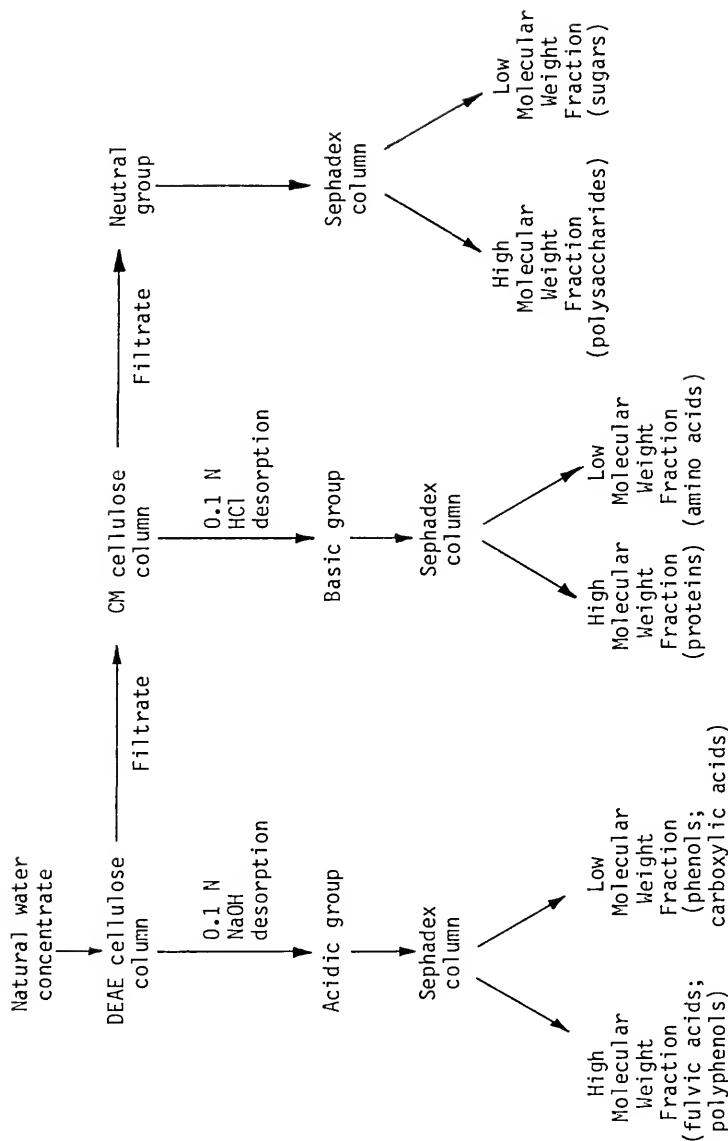


Figure II-6. Scheme for fractionation of dissolved organic matter (Sirotkina et al. 1974).

Leenheer (1981) used nonionic and ion-exchange resins to fractionate organic matter into hydrophobic (base, acid, and neutral fractions) and hydrophilic (base, acid, and neutral fractions) classes. The author claims to have overcome the problems of poor recoveries reported by others when using ion-exchange resins. An extensive cleanup of resins and a detailed desorption procedure were used to recover at least 80% of the adsorbed material.

CHAPTER III
AREAS OF INVESTIGATION, SAMPLING PROCEDURES,
AND ANALYTICAL METHODS

A. Jasper Study

1. Site Description

Basin Swamp, located near Jasper, Florida, was selected as an area to study the efficiency and effects of using cypress strands for tertiary treatment of wastewater during the 2-year period January 1979 through December 1980. This swamp has been receiving wastewater effluent from the City of Jasper since 1914. Raw sewage was released into the swamp from 1914 to 1951; primary treated effluent was released from 1952 to 1972; and secondary effluent has been released since then. Typically, the plant discharges 1150 m³/day (0.3 MGD) into the swamp except during periods of heavy rainfall, when the rate increases to 2200 m³/day (0.6 MGD).

The wastewater that leaves the Jasper sewage treatment plant flows first into an area that is now a marsh (Upper Basin Swamp; Figure III-1). A railroad embankment divides this area into two sections (East Swamp and West Swamp), connected by two culverts. The West Swamp, which receives the effluent directly, is dominated at the northern end by herbaceous plants such as elephant ear (Colocasia esculentum) and pennywort (Hydrocotyle umbellata). Bushes and small trees (e.g., red maple [Acer rubrum]) are more common in the middle and

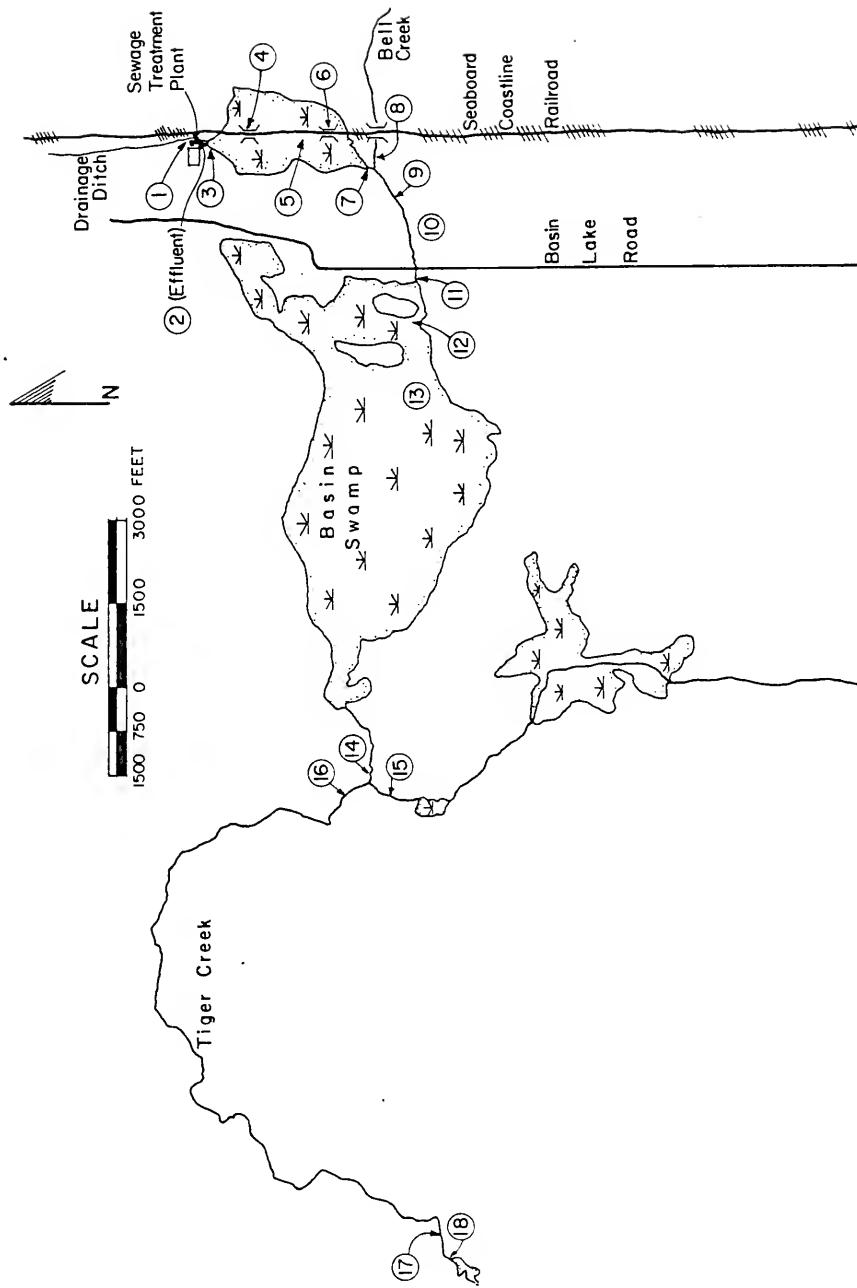


Figure III-1. Surface water sampling stations at study site near Jasper, Florida.

southern end of the marsh, and cattails (*Typha latifolia*) line both the east and west boundaries. Unconsolidated sediments are more than a meter deep in several places.

Surface water from Upper Basin Swamp channelizes and converges with Bell Creek, which flows into Lower Basin Swamp (Figure III-1). Vegetation in Lower Basin Swamp is similar to that described for Upper Basin Swamp, although more cypress trees (*Taxodium distichum*) are present in the downstream swamp than in the upper area. Further downstream, channelized flow continues (Tiger Creek) and surface water flow terminates at a sinkhole (Tiger Sink).

2. Sampling and Analytical Methods

Samples for heavy metals were collected at several points throughout the swamp and along the channelized flow downstream, including the sinkhole into which Tiger Creek flows (Figure III-1). The heavy metal content in surface runoff was measured at the drainage ditch from the City of Jasper (station 1), Bell Creek (station 8), and a tributary of Tiger Creek (station 15). In addition, samples were collected at both the influent and effluent of the sewage treatment plant. Groundwater quality was monitored at the wells shown in Figure III-2.

Surface water samples were collected directly (by hand) in acid-washed plastic sample bottles, and groundwater samples were collected with a hand vacuum pump by drawing water through Tygon tubing into a sample bottle. Prior to sampling the wells, the water in the well pipe was pumped out and discarded so that fresh percolate could be collected. All samples for heavy-metals analysis were filtered through a 1- μm polycarbonate filter and preserved with high purity nitric acid

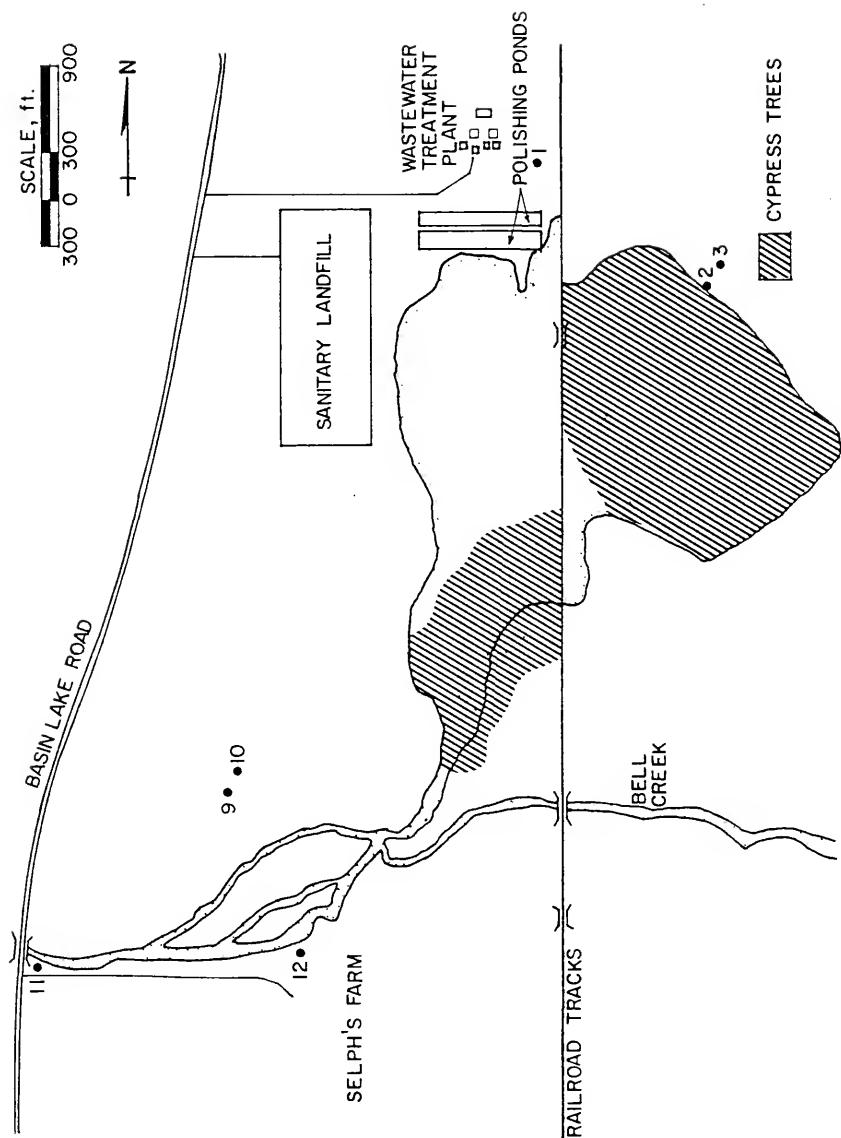


Figure III-2. Locations of groundwater wells at Basin Swamp study site near Jasper, Florida.

(Ultrex) at the rate of 5 mL/L of sample. Cadmium, copper, lead, and nickel were analyzed using a flameless graphite-furnace technique with an atomic absorption spectrophotometer (AAS), whereas manganese and zinc were analyzed by direct aspiration in the flame mode of the AAS. Instrument settings were those recommended by the manufacturer (Varian 1972).

B. Waldo Study

1. Site Description

The City of Waldo, Florida (population ~1000), has been discharging wastewater into a series of cypress wetlands for over 45 years. During that time, the sewage from Waldo has been treated only by a concrete septic tank, and, at most, primary treatment was effected. The septic tank effluent (septage) flowed through a ditch and into a 2.6-ha cypress wetland on the eastern side of a railroad embankment (Figure III-3). After traveling approximately 1.6 km, the surface water flowed through a culvert under the railroad tracks and into a large (2500 ha) cypress swamp, which eventually drains into Lake Altho and ultimately into the Santa Fe River.

The swamp forest is dominated by an even-age stand of pondcypress (Taxodium distichum var. nutans), with a subcanopy of swamp black gum (Nyssa sylvatica var. biflora) and red maple (Acer rubrum) (Nessel 1978). The understory includes a variety of shrubs, vines, and herbs. For average water conditions, the surface water was approximately 0.5–1.0 m in depth. A dense mat of duckweed (Lemna sp.) covered most

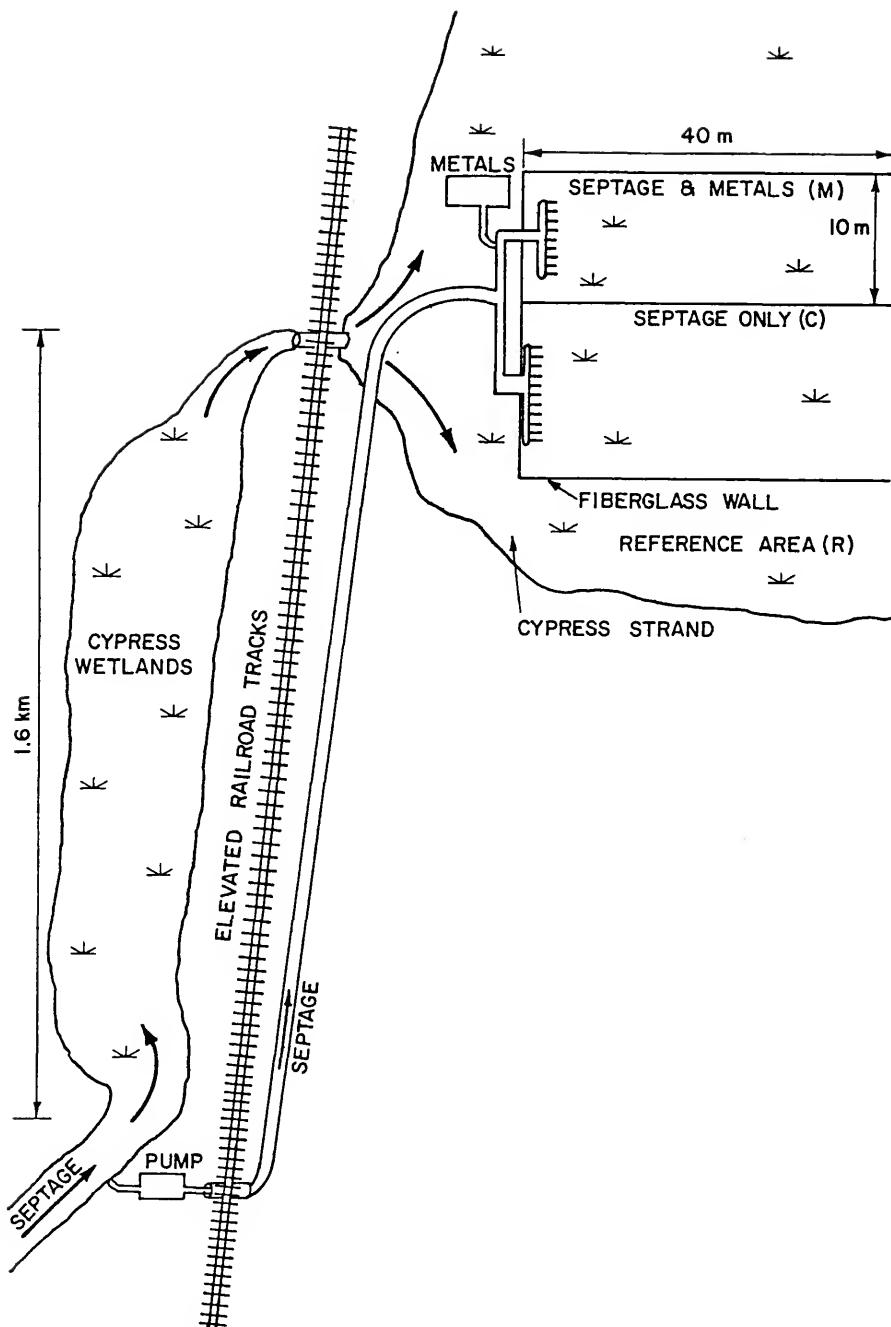


Figure III-3. Map of experimental site at Waldo, Florida.

of the surface water during the warm months, and waterhyacinths (Eichhornia crassipes) grew luxuriantly in some areas.

In order to confine a portion of swamp so that heavy metals could be added and monitored quantitatively, two corridors were constructed in the downstream area of the wetland. This area was thought to be impacted less by antecedant discharge of wastewater than the upstream area, which was closer to the source (Figure III-3). Septage was pumped through plastic pipes to the experimental plots where the wastewater flow was bifurcated, with one plot receiving septage spiked with heavy metals (plot M) and the other plot receiving septage only (plot C). In addition, a reference station was established outside the experimental plots (station R).

Wastewater was pumped at a flow rate between 10 and 40 L/min in each plot, which produced a hydraulic retention time in the plots of approximately 3–5 days. Metals were added using a constant-pressure Mariotte bottle such that the final metal concentration in the wastewater approximated the levels reported for municipal wastewater in New York and Los Angeles (Chen et al. 1974; Klein et al. 1974; see Table II-1), namely: Cd, 10 µg/L; Cu, 50 µg/L; Mn, 100 µg/L; and Zn, 100 µg/L. The chloride salts of each metal were used to make the stock solution.

2. Sampling and Analytical Methods

Surface water samples were collected for metal analyses from March 3, 1981, to April 1, 1981. Subsequent sampling was prevented by severe drought because the amounts of both swamp water and septage were insufficient to continue the experiment. Sampling stations were

located at the sewage discharge pipe and at 10, 20, 30, and 40 m from the discharge point in both the corridor receiving sewage spiked with heavy metals and at the corridor receiving sewage only. Triplicate samples were taken at each station, and in most cases the samples were composited. Samples were preserved with nitric acid (U.S. EPA 1979) and autoclaved (121°C, 2 atm) for 1 h to release colloidal and particulate-bound metals. Copper, zinc, and manganese were analyzed using a Perkin-Elmer model 5000 AAS in the flame mode, and cadmium and some low-level samples for copper were analyzed by the graphite-furnace technique with an AAS. Instrument settings were those recommended by the manufacturer (Perkin-Elmer 1980).

C. Microcosm Study

Twenty microcosms were constructed by adding 800 g of wet peat, a layer of partially decomposed leaves (litter), and 3 L of filtered water to a 4-L jar in a manner simulating the structure of the swamp ecosystem at Waldo (Figure III-4). The peat, litter, and surface water were collected from an undisturbed area in Waldo Swamp and kept at 4°C until needed. After the microcosms were constructed they were purged with a slow stream of nitrogen for 1 month to allow the sediments and water to reach steady state. Several distinct horizontal layers in the sediment were visible through the plastic containers, and apparently the vertical structure of the sediments had been re-established.

Initially, the intent was to vary redox potential (E_h), dissolved organic carbon (DOC), pH, calcium, and iron levels in the microcosms and to observe the correlation of these factors with metal loss.

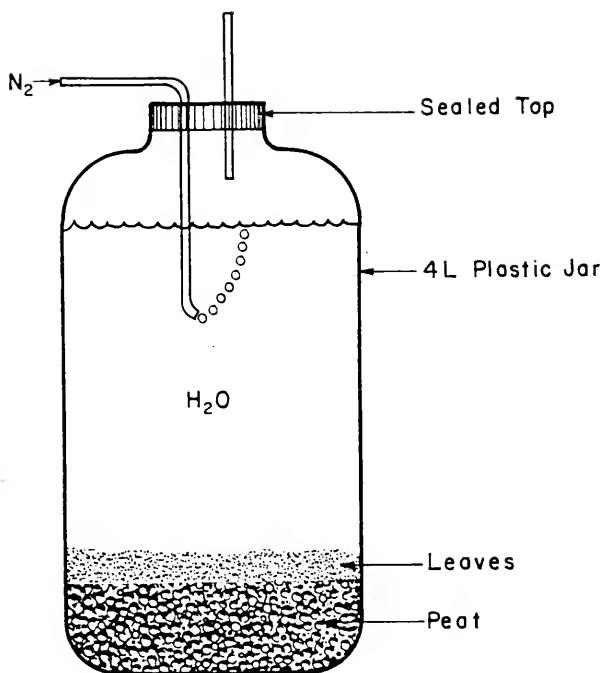


Figure III-4. Illustration of microcosm.

However, E_h was not easily regulated. Control of E_h was attempted by allowing conditions in the microcosms to become reduced and subsequently purging the system with a variable amount of oxygen. This method produced inconsistent levels of E_h , and therefore another approach was taken. The E_h was controlled externally by purging the microcosms with various levels of hydrogen sulfide (H_2S). The three levels of H_2S used in the study were <0.1, 0.5, and 5.0 mg H_2S/L (Table III-1). The lowest value was achieved by purging the systems with nitrogen and enough oxygen to maintain oxidizing conditions (~1–2 mg O_2/L), which were similar to field conditions in Waldo Swamp during the winter months. The other two levels were maintained by bubbling a mixture of nitrogen and H_2S into the water of the microcosms. Levels of E_h were measured during the preliminary experimental work, and at H_2S concentrations of <0.1, 0.5, and 5.0, E_h was +480, -100, and -100 mV, respectively. Although the levels of H_2S and E_h are interdependent, the level of H_2S was varied in the experiment, and hence the factor H_2S is used in subsequent discussions.

The five factors examined in this study—DOC, iron, calcium, sulfide, and pH—were tested at two levels such that all possible combinations of the two levels were made (Table III-1). This factorial design experiment had 32 (i.e., 2^5) different treatments. The intermediate levels were performed in replicate so the variability of the experiment could be determined. The experiment was conducted in two stages using 20 microcosms for each stage. After the microcosms were used once, the water was acidified to pH 2 to desorb metals from surface sites, and the acidified water was replaced with fresh surface water. In addi-

Table III-1. Factors and levels for factorial microcosm experiment.

Factors	Levels		
	High +	Low -	Intermediate 0
DOC (mg/L)	50	17	33
pH	7.0	4.0	5.5
Calcium (mg/L)	40	4.0	22
Iron (mg/L)	5.0	0.3	2.5
Sulfide (mg/L)	5.0	<0.1	0.5

tion, several systems containing only surface water were examined concurrently with the microcosms (which contained both water and peat sediments).

Calcium and iron levels in the microcosms were increased by the addition of the chloride salts. DOC was reduced from the original level of 50 mg/L by passing the water through an exchange column of DEAE-cellulose and mixing with untreated water to obtain the desired level of DOC. The pH was adjusted with HCl or NaOH, and sulfide was increased by continuously bubbling H₂S into the containers. Sulfide concentrations were measured using the fluorescein procedure described by Natusch et al. (1972). The water in the microcosm was gently circulated by a constant stream of nitrogen (2 L/h).

After the conditions were set, the microcosms were spiked with cadmium, copper, zinc, and manganese at levels of 10, 50, 100, and 100 µg/L, respectively. The overlying water was sampled at 24-h intervals, and the levels of metal remaining in solution were determined by atomic absorption spectroscopy. The treatment parameters DOC, iron, and calcium were adjusted at the beginning of each experiment only, whereas the sulfide and pH levels were maintained at constant values throughout the experiment.

D. Analytical Methods for Determining C_L and β'

This section describes the instrumentation and methodology used to evaluate and compare five methods for determining C_L and β'. Three of the methods, ASV, ISE, and fluorescence, are published, and two have received widespread use. The fluorescence method was pub-

lished only recently. Titrations using these three methods were performed according to each published procedure. The other two procedures, ultrafiltration and the competing ligand method, were adapted from studies not directly related to metal speciation in natural waters. For the latter two methods, the development of each procedure is described in this section in addition to the final experimental procedure, because these details are not documented elsewhere.

Evaluation of the ASV complexometric technique used solutions containing a single organic compound such as EDTA, histidine, and Desferal, for which β' for copper has been published. For the comparative study, two colored-water samples from Waldo and Basin swamps and three homopolyamino acids were titrated. The conditions of pH (6.25) and ionic strength (0.1 M KNO₃) were identical for each of the five procedures used in the comparison study.

1. Anodic Stripping Voltammetry

Titrations were performed according to the procedures described by Shuman and Woodward (1973, 1977), except that the stripping step of ASV was performed in the differential pulse (DP) mode rather than the linear (DC) mode. Instrumentation consisted of a PAR model 174 polarographic analyzer using a Kemula-type hanging mercury drop electrode (HMDE). A saturated calomel reference electrode (SCE) was connected to the solution by a 0.01 M KNO₃ bridge. Instrument settings were 50 mV modulation amplitude and 1 s⁻¹ pulse rate. Pseudopolarograms were constructed by the method of Figura and McDuffie (1979) to determine appropriate plating potentials. Cadmium was plated at -0.8 V versus SCE and scanned at 5 mV/s. Copper was plated at -0.3 V versus SCE and

scanned at 2 mV/s. Zinc was plated at -1.2 V versus SCE and scanned at 5 mV/s. The initial potential was applied for 60–300 s with stirring, followed by a 15-s quiescent period. Analyses were performed with 50-mL samples containing 30–50 mM sodium acetate buffer, 10–100 mM KNO₃, or 1 mM KNO₃ (Desferal). All electrolytes were purified by electrolysis.

Cyclic voltammetry was performed using an HDME in the DC mode. The scan rate was 50 mV/s; solution conditions are described in the results section.

Nitrogen gas, used to deoxygenate and stir each test solution, was regulated by an in-line ball flowmeter. The pH was adjusted with NaOH after deoxygenation and solution pH was checked at the end of each titration to assure constant pH. A gel-filled pH electrode was used so that chloride contamination of samples was avoided.

Solutions were prepared from distilled and deionized (Milli-Q) water, and all chemicals were reagent grade except Desferal (Ciba-Geigy) and the polyamino acids (Miles). Metal solutions were added in μ L quantities to avoid dilution, and prior to analysis, samples were equilibrated 15 min after each addition of metal. Rapid equilibration of each ligand with metal was verified by analyzing solutions that had equilibrated in quartz test tubes up to 3 h. In all cases, equilibration was achieved within 15 min. Analyses were duplicated after each metal addition, and each organic compound was titrated at least twice. Linear portions of titration curves were modeled by least squares linear regression.

a. Theoretical considerations for determining C_L and β' by ASV.

With each addition of metal M, the reaction with the ligand L proceeds according to:



The concentration of metal not bound to L, which is in equilibrium with M_aL_b , is determined by ASV after each addition of metal. A plot of the resulting peak current, i_p , versus the concentration of metal added (Figure III-5), can be used to determine the available ligand concentration, C_L , and, ultimately, the conditional stability constant, β' , of the metal-ligand complex.

Shuman and Woodward (1977) derived the following equation to determine the conditional stability constant of the M_aL_b complex:

$$i_p \approx \left(\frac{S_u}{\beta'}\right)^{1/a} \left[\frac{(C_M/a)^{1/a}}{\left(C_L - \frac{b}{a}C_M\right)^{b/a}} \right]. \quad (3.2)$$

The symbol C_M is the concentration of metal added, C_L is the available ligand concentration (determined graphically), S_u is the slope of the upper region of the titration curve, and β' is the conditional stability constant. In the case of large organic molecules, C_L is interpreted as the molar concentration of the individual functional groups that act independently as ligands.

Given eqn. 3.2, a plot of i_p versus

$$\left[\left(\frac{C_M}{a} \right)^{1/a} \right] / \left[\left(C_L - \frac{b}{a} C_M \right)^{b/a} \right]$$

in the region of the titration curve well before the equivalence point produces a slope of $(S_u/\beta')^{1/a}$. The conditions stated by Shuman

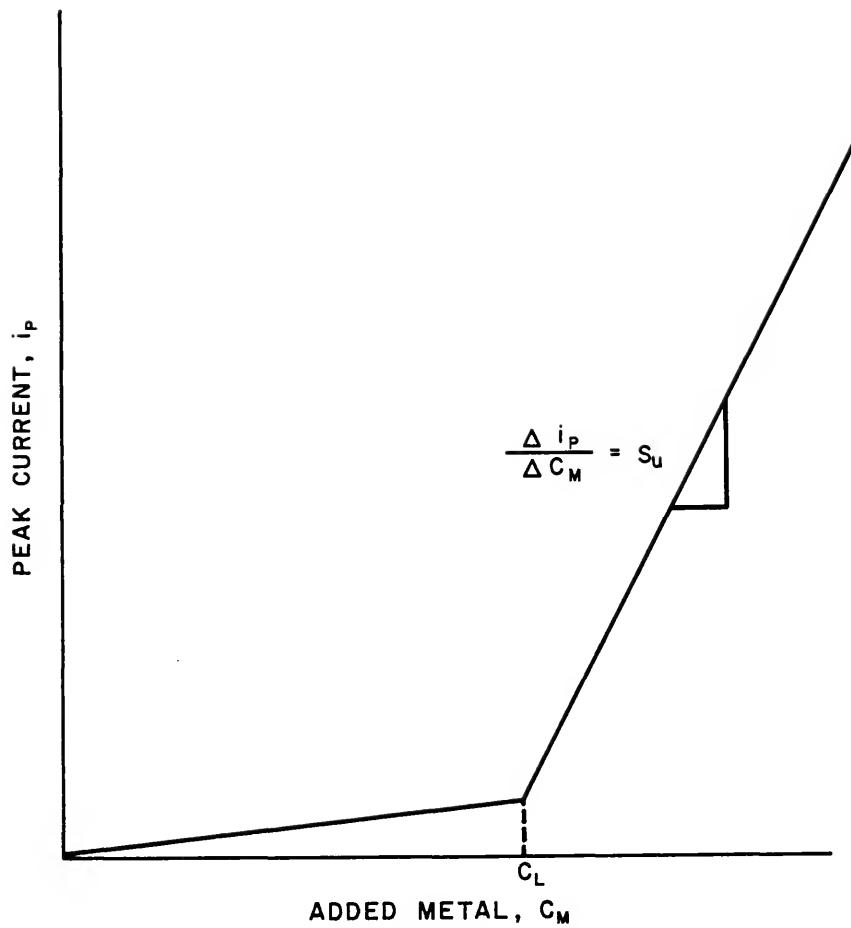


Figure III-5. Theoretical ASV titration curve of ligand with metal.

and Woodward (1977) when they derived eqn. 3.2, require $C_M < C_L$, so only the values obtained from the first few additions of titrant are plotted for this curve. By inserting reasonable integers for a and b, and plotting i_p versus

$$[(\frac{C_M}{a})^{1/a}] / [(C_L - \frac{b}{a}C_M)^{b/a}]$$

several curves are obtained for each titration. The curve most closely approaching a straight line will indicate the stoichiometry of reaction 3.1. The slope of the most nearly linear line, S_L , is equal to $(S_u/\beta')^{1/a}$ and the ratio of S_u/S_L can then be used to determine β' , the conditional stability constant of the M_aL_b complex.

Several assumptions are required for this procedure to work:

1. the concentration of available ligand was assumed to be in excess prior to the addition of the metal titrant;
2. metals bound to ligand of interest more weakly than the titrant metal are displaced by titrant metal;
3. peak current, i_p , is proportional to the concentration of metal not bound to the ligand of interest; and
4. the metal-ligand complex does not kinetically dissociate during the plating step.

Assumptions (3) and (4) are addressed by this research and discussed in Chapter IV.

In addition to the method of Shuman and Woodward (1977) to determine conditional stability constants, the ASV data were handled by the Scatchard procedure, which is discussed at the end of this chapter (section III-D-6).

2. Ion-Selective Electrode

Complexometric titrations of swamp samples and model compounds were performed using an ion-selective electrode (ISE) in a manner similar to that described for the ASV procedure, except that a copper-ISE was used to detect uncomplexed copper, rather than a mercury electrode. Samples (50 mL) were added to a polyethylene beaker and were maintained at 25°C in a constant temperature bath. Potentials were measured with an Orion Cu-ISE (model 94-29) and a double junction reference electrode containing KNO_3 in the outer reservoir. The pH was maintained at 6.25 ± 0.05 by purging with a mixture of CO_2 and N_2 gases, and solutions were continually stirred with a silanized glass paddle connected to a stirring motor. Ionic strength was adjusted to 0.1 M with KNO_3 , and copper was added in μL quantities to avoid dilution. The electrode potential was monitored continually with a strip-chart recorder to ensure that equilibrium had been obtained at each titration point. Between samples, the Cu-ISE was soaked in a solution of 10^{-3} M EDTA for 10 min to remove excess copper from the electrode surface. The system was calibrated frequently by titrating deionized water adjusted to the same ionic strength and pH as for the samples. The activities of ionic copper were calculated by:

$$\text{pCu}^{+2} = \text{pCu}_t + (E_{\text{std}} - E_{\text{sample}})/s \quad (3.3)$$

where pCu^{+2} and pCu_t are the negative logs of copper ion activity and total copper concentration, respectively, E_{std} and E_{sample} are the potentials (mV) of the standards and samples at identical pCu_t , and s is the slope of the standard calibration curve in mV.

3. Fluorescence Quenching

The method described by Saar and Weber (1980) was used to determine C_L and β' for samples using copper as titrant. Samples were placed in test tubes, ionic strength was adjusted to 0.1 M using KNO_3 , and copper was added to each solution in increasing amounts using micropipets to avoid appreciable dilution. Subsequently, pH was adjusted to 6.25 using HCl or NaOH. Relative fluorescence of each sample was measured using an Aminco Model SPF125 at an excitation wavelength of 350 nm and an emission wavelength of 450 nm. Solutions containing increasing levels of copper were analyzed until the copper concentration was such that flocculation occurred.

The concentration of copper bound (Cu_b) at each titration point was calculated using the equation:

$$\text{Percent Quenched}/57 = \bar{V} = (Cu_b)/(L_T) \quad (3.4)$$

where (L_T) is the total ligand concentration and 57 is an empirically derived coefficient (Saar and Weber 1980). Therefore, at any point in the titration, the concentration of uncomplexed copper (Cu_f) was calculated by:

$$(Cu_f) = (Cu_t) - (Cu_b) \quad (3.5)$$

where (Cu_t) is the total (analytical) concentration of copper in solution. These data were treated further by the method of Scatchard (1949) to produce conditional stability constants for the copper complexes (see section III-D-6).

4. Continuous Ultrafiltration

Several studies have used the process of ultrafiltration for quantifying metals associated with high-molecular-weight organics in natural waters (Gjessing 1971; Guy and Chakrabarti 1976; Schindler et al. 1972; Hoffman et al. 1981). The membrane filters allow low-molecular-weight compounds and ions to pass through the filter, whereas high-molecular-weight organics and the metals associated with them are impermeable to the membrane and are retained. The most common application of ultrafiltration to natural water studies has been the determination of metal distribution by filtering a sample at a single metal and ligand concentration. For this approach, filtration must be repeated if different concentrations of metal and ligand are to be evaluated.

One application of ultrafiltration that obviates the necessity of repeating the experiment for each concentration of binding component was reported for binding of microsolutes (e.g., steroids, oligopeptides) to large proteins (Ryan and Hanna 1971). The method is a continuous ultrafiltration technique in which a pressurized reservoir containing a solution of the binding component of interest is continuously fed into a stirred ultrafiltration cell, which contains the high-molecular-weight organic matter initially with no binding component (Figure III-6). By collecting discrete fractions of effluent, the free or unbound concentration of microsolute can be evaluated for various concentrations of total microsolute. The level of bound microsolute can be calculated for each fraction collected by performing a simple mass balance, since the total quantity of microsolute added at any point during the titration is known.

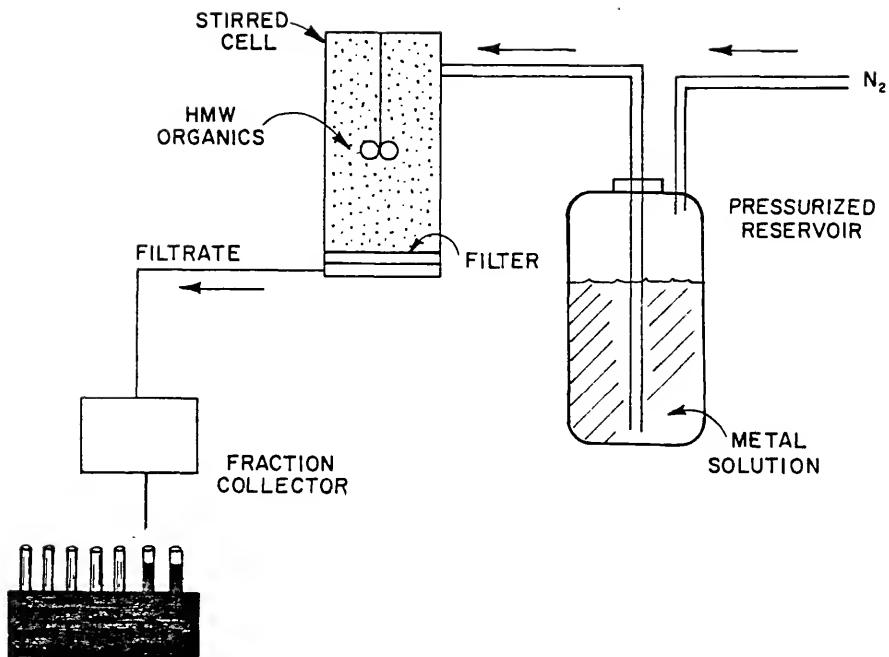


Figure III-6. Diagram of apparatus for method of continuous ultrafiltration.

The continuous ultrafiltration procedure reported by Ryan and Hanna (1971) was adapted in this study so that the extent of metal binding with organic matter could be evaluated. However, several difficulties appeared during the development of the method. The type of filtration membrane was found to be important. The small pore-size filters have more ionic character than the large porosity filters, and solute interaction with ionic membranes produced erratic results. On the other hand, with increasing porosity, retention of organic matter diminished. The Amicon UM-10 (10,000 dalton) membrane was found to be the best compromise. The molecular weight cutoff values assigned to the filters are approximate and apply to globular proteins and sugars. Thus, membrane rejection is not related solely to molecular weight, since retention is a function of both size and charge of solute.

Another parameter found to control the success of this procedure was the flow rate through the membrane. At fast flow rates (0.5–1.0 mL/min) and with a purely inorganic matrix, the concentration of metal in the retentate eventually exceeded the concentration of metal in the feed solution, apparently due to partial rejection of ionic metal by the membrane. A flow rate of 0.2 mL/min alleviated the problem of enhanced concentrations of metal in the retentate, but even at that flow rate, the metal concentration in the filtrate was lower than that in the retentate for purely ionic solutions. Ionic metal was binding to the membrane and causing low results. It was discovered that the ionic strength of the solution influenced the extent of metal adsorption to the filters, and subsequently, all solutions were adjusted with KNO_3 to 0.1 M, the lowest level found to prevent metal loss to the membrane.

After the above problems were rectified, the samples from Waldo Swamp, Basin Swamp, and several model compounds were analyzed using the following procedure. Samples were filtered through 1- μm pore size Nucleopore membrane filters, and 70 mL of the filtrate was placed above an Amicon UM-10 ultrafiltration membrane in a 70-mL-capacity filtration cell (Amicon Model 52). Each sample was rinsed with a solution of 0.1 M KNO_3 at pH 6.25 to remove the nonretained compounds. During the rinsing process, great care was taken to ensure that the sample was not contaminated with metal from a previous titration. Several membranes were dedicated to rinsing samples and were replaced during metal titrations. The cell and tubing were rinsed with dilute acid prior to adding samples or solutions. After rinsing, the sample was transferred from the cell to an acid-washed bottle. The membrane was changed and equilibrated with a dilute solution of the metal of interest for 1 h. Subsequently, the membrane was rinsed with 15 mL of metal-free buffer and the sample was added to the cell. The reservoir, which contained the solution of metal (and 0.1 M KNO_3 at pH 6.25), was connected to the ultrafiltration cell, and nitrogen gas was applied at a pressure of 2 atm. The sample was stirred continuously, and effluent was collected in 3–8-mL fractions with a Gilson GME automatic collector. After collection, samples were acidified with nitric acid and analyzed by atomic absorption spectroscopy (Perkin-Elmer Model 5000).

The data generated with the continuous ultrafiltration technique were used to calculate the quantity of metal bound, M_b , and metal not bound, M_f , to the retained ligand for each fraction collected by using a simple balance of materials,

$$M_b = M_{\text{added}} - M_f - M_{\text{out}} \quad (3.6)$$

where M_{added} and M_{out} are the total amounts of metal added to the cell and metal in the cell effluent, respectively. Equation 3.6 can be expanded to:

$$M_b = V_{\text{out}}(M_0) - V_{\text{cell}}(M_e) - M_{\text{out}} \quad (3.7)$$

where V_{out} is the volume of effluent collected, (M_0) is the initial metal concentration (in the feed solution), and (M_e) is the measured metal concentration in the effluent (which is equal to the free metal concentration in the cell at the time the fraction was collected). Therefore, the amount of metal bound to the organic ligand at any point throughout the titration can be determined by eqn. 3.7. A programmable calculator was used to reduce the experimental data such that for each fraction collected, both free and bound metal were computed. These data were treated further by the method of Scatchard to determine conditional stability constants for the metal-organic complexes (see section III-D-6).

To perform the continuous ultrafiltration titration accurately and to compute the levels of metal bound and not bound to retained organic matter by the methods described, the following conditions are necessary:

1. the ligand of interest is retained by the membrane;
2. the cell contents are completely mixed;
3. the effluent metal concentration is equal to the unbound metal concentration in the cell;
4. the cell volume remains constant; and
5. the effluent is collected in accurately measured volumes.

5. Competing Ligand/Differential Spectroscopy

This procedure involves adding a competing ligand (A) that has a known stability constant with the titrant metal to a solution of the metal (M) and ligand (L) of interest, and thus establishing the following equilibria:



By quantifying either A or MA, and knowing the analytical concentrations of M, L, and A, the value of the conditional stability constant for the ML complex can be determined. Details of such a calculation are presented later in this section.

Several organic compounds were examined for use as competing ligands in this study. Of the ligands examined, most absorbed light in the regions below 300 nm, which is an area that naturally occurring organics absorb strongly. Scans of aqueous solutions of nitrilotriacetate, citric acid, phenylalanine, and histidine revealed only negligible absorption between 300 and 600 nm. The addition of copper to these ligands did not affect any of their absorption spectra at wavelengths above 300 nm. However, salicylate (anionic form of *o*-hydroxy benzoic acid) was found to absorb light strongly at 291 nm, and the addition of copper caused a bathochromatic shift (i.e., to longer wavelengths) in the absorption band of salicylate (SAL) to a wavelength of 320 nm at maximal intensity. The concentration of the Cu-SAL complex was found to be proportional to the absorbance at 320 nm. Because

salicylate, at the concentrations used, absorbed appreciably at 320 nm, this background absorbance was subtracted optically by performing differential spectroscopy; namely, the sample without added metal was placed in the reference path of the spectrophotometer, and the sample with added metal was placed in the sample path. Thus the instrument measures the difference between the two samples, which in this case is the absorbance due to CuSAL.

Sample preparation consisted of pipeting 50.0 mL of sample into each of several 125-mL flasks and adding 0.50 mL of 10^{-1} M salicylate. The ionic strength was increased to 0.1 M with KNO_3 and copper was added in various quantities to each flask. The pH was adjusted to 6.25 ± 0.05 using small aliquots of NaOH or HCl to minimize dilution. Sample containing no additional copper was placed in the reference cell (either 1-cm or 4-cm pathlength) of a Perkin-Elmer Model 552 spectrophotometer and samples with copper added were placed in the sample cell. Absorbance was measured at 320 nm.

Absorbance values were standardized by titrating a solution of 10^{-3} M salicylate with copper. The concentration of CuSAL at each level of total copper concentration was calculated by computer program (MINEQL), and a plot of absorbance versus CuSAL concentration is presented in Figure III-7. The lower limit of this procedure using a 4-cm cell is $2.0 \cdot 10^{-7}$ M CuSAL. Measurement of lower concentrations was attempted with a 10-cm cell, but total absorbance was beyond the maximum absorbance measurable with the instrument.

The absorbance values obtained for samples were converted to concentrations of CuSAL using the data in Figure III-7. Hence, for the competing equilibria,

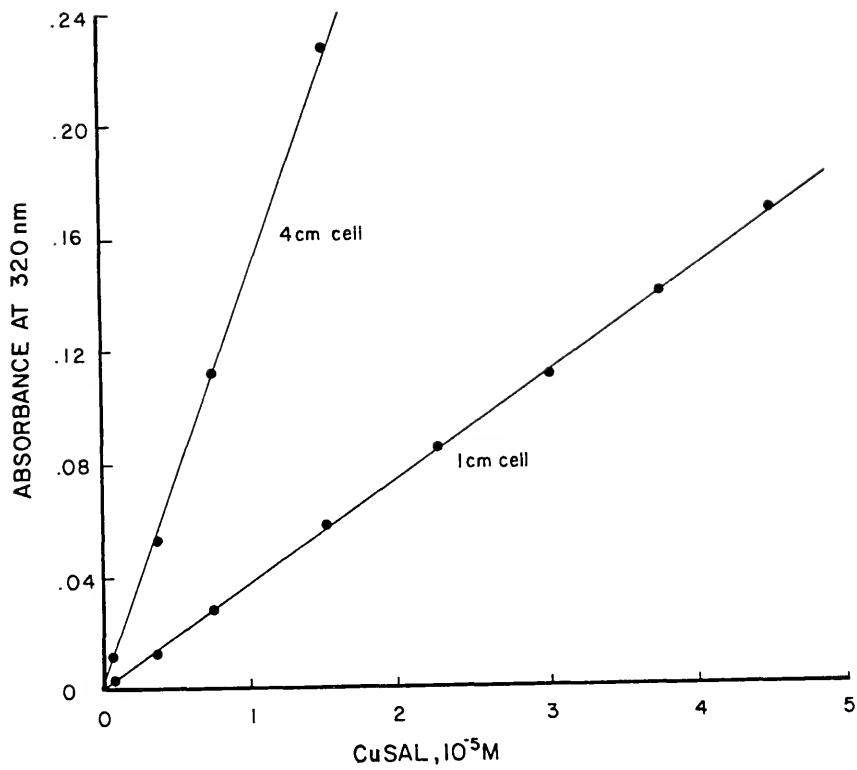


Figure III-7. Calibration curve for competing ligand/differential spectroscopy method.

$$\text{CuL} = \text{Cu}_f + \text{L} \quad (3.10)$$

$$\text{Cu}_f + \text{SAL} = \text{CuSAL} \quad (3.11)$$

where L is the ligand of interest, Cu_f is uncomplexed copper, and SAL is salicylate, equilibrium concentrations of Cu_f , CuL, and L were calculated by using the mass balance of each component, the value of β'_{CuSAL} , and the empirically derived concentration of CuSAL. The mass balance equations for this experiment are:

$$\text{Cu}_T = (\text{Cu}_f) + (\text{CuL}) + (\text{CuSAL}) \quad (3.12)$$

$$\text{SAL}_T = (\text{SAL}) + (\text{CuSAL}) \quad (3.13)$$

$$\text{L}_T = (\text{CuL}) + (\text{L}) \quad (3.14)$$

where the parentheses indicate aqueous concentrations and the subscript T indicates the total concentration of each species. The conditional stability constant for the Cu-SAL complex was calculated from the thermodynamic stability constant (Sillen and Martell 1971) by the method of Ringbom (1963) (q.v., section IV-A). The value of β'_{CuSAL} at pH 6.25 was calculated to be 10^{3.5} and thus

$$\beta' = 10^{3.5} = \frac{(\text{CuSAL})}{(\text{Cu}_f)(\text{SAL})}. \quad (3.15)$$

The empirical concentration of CuSAL for each titration point was inserted into eqns. 3.13 and 3.15, and (Cu_f) was calculated by simultaneously solving the two equations. Subsequently, (CuL) was determined using eqn. 3.12. These data were treated further by Scatchard analysis to determine β' .

6. Theoretical Considerations for Determining
 β' by Scatchard Analysis

Conditional stability constants, β' , can be estimated by using the method of Scatchard (Scatchard 1949; Guy and Chakrabarti 1975; Mantoura and Riley 1975), in which it was assumed that

$$\beta' = (M_b)/(M_f)(nL_T - M_b) \quad (3.16)$$

where (M_b) and (M_f) are concentrations of metal bound and not bound to ligand, L_T is the total ligand concentration, and n is the number of binding sites per ligand molecule. Equation 3.16 can be rearranged to

$$(M_b)/(L_T)(M_f) = \beta'[n - (M_b)/(L_T)]. \quad (3.17)$$

By substituting \bar{V} for $(M_b)/(L_T)$, the final form of the equation becomes

$$\bar{V}/(M_f) = \beta'(n - \bar{V}). \quad (3.18)$$

Thus a plot of $\bar{V}/(M_f)$ versus \bar{V} will produce a curve with slope $-\beta'$, and n can be determined from the intercept value. This data analysis has been attributed to Scatchard, and a plot of $\bar{V}/(M_f)$ versus \bar{V} is termed a Scatchard plot.

Originally, the method was intended for use with pure compounds containing one or more distinct binding sites. Thus for a single ligand with one class of binding site, a titration with a metal will produce a straight line with slope $-\beta'$ and an intercept of n on the \bar{V} axis. However, the typical curve obtained by titrating environmental

samples with metal is asymptotic due to the variable strength of metal binding. Many researchers who use Scatchard plots for determining β' for metals with naturally occurring organics classify the sites into two categories with one "strong" site and one "weak" site (Figure III-8). However this approach is too simplistic to characterize the variety of complexes expected in environmental samples. For the data obtained here, subtraction of the "strong" site from the "weak" site produced plots that remained curved. The slope of the "strong" site is too steep to have much effect on the remainder of the titration curve. Therefore, the conditional stability constant data generated here were treated as a continuum, and the method commonly used to correct the plots for contributions from the "strong" and "weak" sites was not performed. Instead, the curves were divided into sections of \bar{V} , and the data were fitted to a straight line. The ranges of \bar{V} were selected by visually sectioning the Scatchard plot from the method that produced the widest range of values (ISE method). Three segments were used to fit the experimental data. Nonetheless, the data are curvilinear, and the only intent of fitting segments of the data to straight lines was so that a comparison could be made among the five analytical procedures used to determine the levels of complexed and uncomplexed metal.

E. Chemical Characterization and Fractionation of Sewage and Swamp Water

Swamp water samples were collected in 5-L quantities from undisturbed areas in Waldo Swamp and Basin Swamp. Secondary sewage effluent

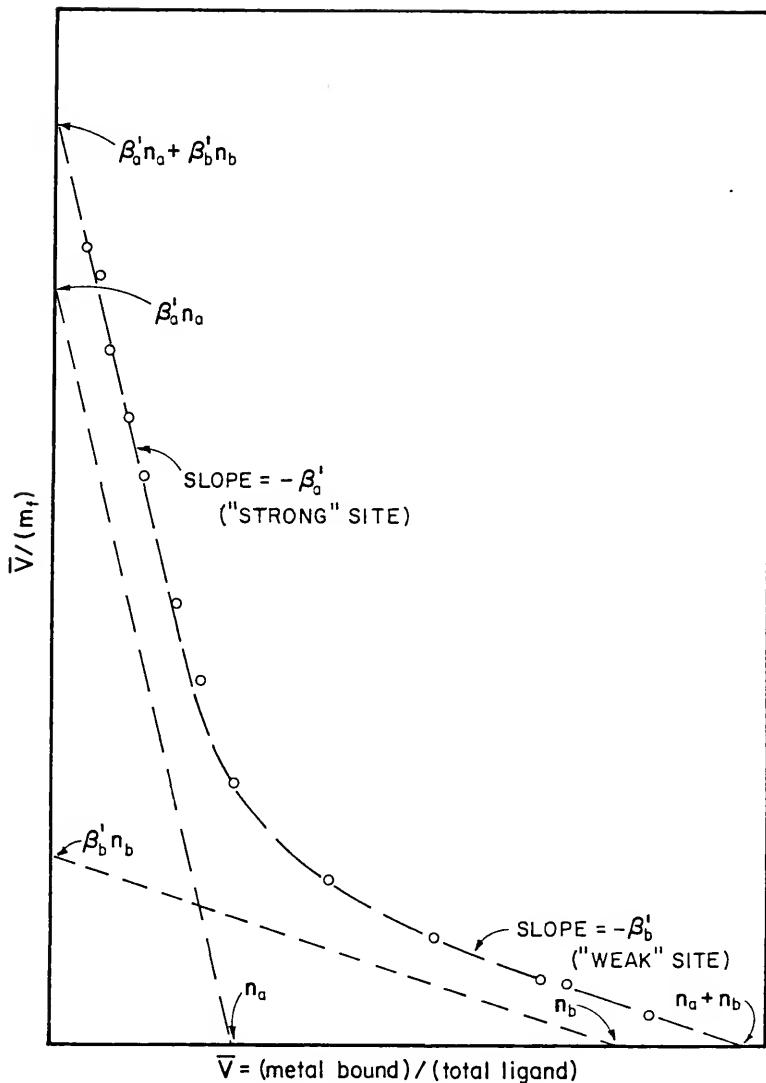


Figure III-8. Theoretical Scatchard plot for titration of organic matter with metal. Dashed lines are resolved contributions (assuming that only two classes of binding occurred).

from the Jasper sewage treatment plant was obtained from the outfall of the oxidation pond that flows directly into Basin Swamp, and septage was collected from the effluent of the septic tank that received sewage from Waldo, Florida. All samples were returned to the laboratory and filtered through a 0.5- μm precombusted (450°C) Reeve Angel 984-H glass fiber filter. Samples were preserved at 4°C until needed.

The two sewage samples were treated further to reduce the carbonate and ammonium concentrations. Carbonate and copper form an ion pair complex that is not measured by ISE. Thus copper-organic complexation is difficult to assess for samples containing appreciable levels of carbonate. Ammonium was removed from samples because the fluorescence procedure for quantifying amino acids is reactive to ammonium, although to a lesser degree than for amino acids. Therefore, the pH was lowered to 4, and the sewage samples were purged with dry nitrogen for 3 h at 35°C to remove carbonates. Subsequently, the pH was increased to 9.5 and purged with dry nitrogen for 3 h at 35°C to remove ammonium.

The inorganic composition of each sample was quantified by determining concentrations of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and the dissolved hydrolyzable amino acid nitrogen (DHAAN). DOC was analyzed by direct injection using a Beckman Model 915 Total Organic Carbon Analyzer equipped with a Model 865 Infrared Analyzer (APHA 1981). Organic nitrogen was determined after Kjeldahl digestion by the automated phenate method (U.S. EPA 1979). The amino acid nitrogen was determined by the fluorescamine technique (Udenfriend 1972) before and after hydrolysis. Samples to be hydrolyzed were placed in precombusted (450°C) ampules to which an equal volume of 12 N HCl was added. The ampules were then purged with dry

nitrogen, sealed, and autoclaved (120°C) for 1 h. After cooling, the ampules were opened and evaporated (45°C) to dryness in a vacuum desiccator. The hydrolysate was quantified by the fluorescamine method using an Aminco Model SPF125 spectrofluorometer.

Sewage samples were fractionated by ultrafiltration using Amicon Diaflo ultrafiltration membranes (UM-02 and UM-10) and a magnetically stirred Amicon Ultrafiltration Cell (Model 52), under nitrogen pressure of 2 atm. Before adding samples, each filter was flushed with 200 mL deionized water to remove contaminants.

The swamp water samples were fractionated using diethylaminoethyl (DEAE) cellulose, which is an anion exchange material. DEAE cellulose (Bio-Rad) was prepared by the following procedure: the exchanger was soaked in 1 M HCl, filtered, rinsed with deionized water, and then soaked in 1 M NaOH, filtered, and rinsed with deionized water. This process was repeated five times. The purified exchanger (15 g) was poured into a 2.5 cm by 25 cm glass column and allowed to settle. The column was rinsed with 100 mL of 0.01 M NaCl at pH 7, and 1-L samples were applied individually to the column. The retained organics were desorbed by gradient elution using 0.1 M Prideaux buffer (0.1 M in phosphoric, acetic, and boric acids) and 1.0 M NaCl. Subsequently, an increasing pH gradient was used (0.1 M Prideaux buffer and 0.2 M NaOH). The Prideaux buffer with NaOH provides a nearly linear pH gradient up to pH 12 (Curtis et al. 1981). Following desorption, the recovered organics were desalted using ultrafiltration with a continuous flow of deionized water.

Copper binding experiments using complete and fractionated samples were performed using the methods of continuous ultrafiltration and ISE.

Experimental details of these procedures are described in section III-D.

F. Copper Binding by Peat Extract

1. Extraction

Waldo peat was extracted using the method of Randhawa and Broadbent (1965), as follows: 200 g of wet A₁ horizon was mixed with 400 mL of 0.1 N HCl and shaken for 1 h. Supernatant was decanted and discarded. Eight hundred milliliters of 0.5 N NaOH solution was added, and the contents was shaken for 4 h. One hundred milliliters of extract was diluted to 2 L and filtered through Whatman 41 paper. The pH was adjusted to 5 by additions of acetic acid, resulting in an additional 20% dilution. Aliquots of this solution were filtered through 0.45 μm Millipore filter paper prior to use.

2. Molecular Weight Fractionation

Gel filtration chromatography (GFC) was performed with Sephadryl, a Sephadex gel with a wide fractionating range (250,000 to 5,000 M.W. as globular proteins). The preswollen gel was added to a 2 x 100-cm glass column and rinsed at 40 mL/min for 4 h with 1 M Tris buffer (pH = 8.6). Operating flow rate was 17 mL/min, and 5-mL fractions were collected automatically by a Gilson fraction collector. The column was rinsed thoroughly (3 times) after each injection. Blue dextran 2000 (M.W. = 2×10^6) and KCl were used to determine void volume (V_0) and total volume (V_t), respectively. Chromatograms of extract and reinjected fractions were produced by monitoring UV absorbance at 280 nm.

Ultrafiltrations were performed using Amicon Diaflo membranes and a magnetically stirred Amicon Ultrafiltration cell (Model 52) under nitrogen pressures of 1.1–2 atm. A 50-mL aliquot of extracted organic matter was added to the cell containing an XM-50 (50,000 dalton cutoff) or XM-300 (300,000 dalton cutoff) membrane. After 25 mL of sample was filtered, the pressure was reduced and the retained portion of the sample was collected for analysis. The organic content of the filtered and retained fractions was quantified spectrophotometrically at 460 and 280 nm.

3. Binding Capacity and Stability Constant Determinations

The ion-exchange method reported by Crosser and Allen (1976) was used to measure the copper binding ability of the peat extract. Dowex AG 50W-X8 strong cation exchange resin (200–400 mesh) was prepared by adding 10 M NaOH to the resin until pH remained neutral and was followed by batchwise equilibration with 0.02 M acetate buffer. Resin was suction dried and 1.0-g amounts weighed into each of 20 250-mL flasks. Then 50 mL of 0.02 M acetate buffer (pH = 5.9) was added to each flask, and 1 mL of peat extract was added to each of the 10 designated flasks. All flasks were shaken to equilibrate for 4 h at 25°C. Copper (as sulfate) was added with micropipettes to yield Cu concentrations ranging from 0 to 6.3×10^{-4} M in both control and ligand-containing flasks. Flasks were shaken an additional 17 h. Total soluble copper was determined by atomic absorption spectrophotometry (Varian model 1200).

Additionally, the copper complexing capacity of the extracted organic matter and the conditional stability constant of the copper-

organic complex were investigated by ASV using the methods of Chau et al. (1974) and Shuman and Woodward (1977). Instrumentation and settings were those described previously (section III-D-1) except that a scan rate of 5 mV/s was used. Copper was plated at -0.4 V versus SCE for 75 s (60 s stirring, 15 s quiescent) prior to stripping. Analyses were performed on 50-mL aliquots of sample extract (which was diluted 1:10) to which 50 μ L of 6-M acetate was added to provide a 6- μ M supporting electrolyte at a pH of 5.9. The solution was deaerated 15 min with dry nitrogen to remove oxygen prior to analysis. Copper was added sequentially and analyzed until a complete titration curve was obtained.

CHAPTER IV
EVALUATION AND COMPARISON OF METHODS
FOR DETERMINING C_L and β'

The first section of this chapter is the result of an evaluation of the ASV titrimetric method for determining metal binding capacity, C_L , and conditional stability constants, β' , using model compounds such as EDTA, Desferal, and histidine. The subsequent sections describe a comparison of five methods to determine C_L and β' using colored water samples from Waldo and Basin swamps, and three homopolyamino acids—polyaspartic acid, polyarginine, and polyalanine. Titrations of surface water samples were performed with aliquots from composite samples, which contained 50 and 25 mg C/L for Waldo and Basin swamps' samples, respectively. For comparative purposes, all Scatchard calculations were performed by using the same molar concentration of ligand— 10^{-4} M (Waldo Swamp) and $5 \cdot 10^{-5}$ M (Basin Swamp). The Scatchard analysis weights β' according to the molarity of binding sites (not molarity of total ligand), and thus the final value of β' is independent of the total molar value attributed to a given ligand concentration. The polyamino acids were homopolymers with molecular weights of 13,900 (polyarginine), 3,900 (polyalanine), and 5,400 (polyaspartic acid).

Because a comparison among the five procedures was intended, identical conditions were used for each method; namely, pH 6.25 and ionic strength 0.1 M KNO₃. The pH was selected as the best compro-

mise between the increasing concentrations of inorganic complexes of metal at higher pH and decreasing organic complexation of metals at lower pH. The ionic strength of 0.1 M KNO_3 was used because it was the minimum level found to prevent adsorption to ultrafiltration membranes.

A. Evaluation of the ASV-Complexometric Titration

The ASV technique has been used widely to determine C_L and β' for natural waters (Chau and Lum-Shue-Chan 1974; O'Shea and Mancy 1976; Smith 1976; Shuman and Woodward 1977; Sugai and Healy 1978; Shuman and Cromer 1979; Srna et al. 1980; Tuschall and Brezonik 1980; Hoffman et al. 1981), but surprisingly no evaluation of the method has been reported using ligands with known stability constants with copper. To date, the only published validation or calibration of the procedure was reported by Shuman and Woodward (1973) who titrated ethylenedinitrilo-tetraacetic acid (EDTA) with cadmium and concluded that the procedure produced accurate results. However, copper is commonly used to titrate natural water samples because it forms much more stable complexes with most organic ligands than does cadmium (Irving and Williams 1948). Because of the discrepancies observed between the ion-exchange technique and the ASV titration in the determination of C_L and β' (q.v., Chapter V), a systematic evaluation of the ASV procedure appeared necessary to determine its validity.

One of the requisite criteria for performing the titrimetric ASV procedure is that the metal-organic complex be nonreducible at the mercury electrode at a potential significantly separated from the

reduction of ionic metal (Shuman and Woodward 1973). Consequently, ligands that form strong complexes with copper were sought so that β' could be determined experimentally and compared to existing literature values. Figure IV-1 illustrates the structures of the organic compounds selected for titration with copper and analysis by ASV.

The first compound titrated was EDTA, and it became apparent why β'_{CuEDTA} had not been determined previously by this method. For solutions of EDTA (no copper added), a broad peak was observed at 0.0 V versus SCE, which is the same potential at which copper is oxidized. Originally, the peak was believed to be caused by contamination of the EDTA or buffer solutions with copper, and all solutions were purified by electrolysis. However, the peak remained. Further investigation showed that this peak decreased in size as EDTA was titrated with copper up to a Cu:EDTA ratio of 0.4 (Figure IV-2), and subsequently a peak at 0.0 V versus SCE increased in size with further addition of copper. Apparently the initial peak was due to the cathodic shift in the oxidation of mercury by EDTA, and the subsequent peak (at Cu:EDTA ratios above 0.4) was due to reducible copper. In addition to copper, several other metals were found to suppress the interfering mercury peak; namely, lead, nickel, cadmium, zinc, and calcium. Therefore a metal that would suppress or mask the interfering peak was added to the EDTA solution so that the initial copper peaks could be quantified accurately. Calcium was selected since it forms a weaker EDTA complex than do most of the heavy metals.

Because the ASV procedure produces a conditional stability constant (β') and most literature values are thermodynamic stability constants (β), one must be converted to the other so that a direct

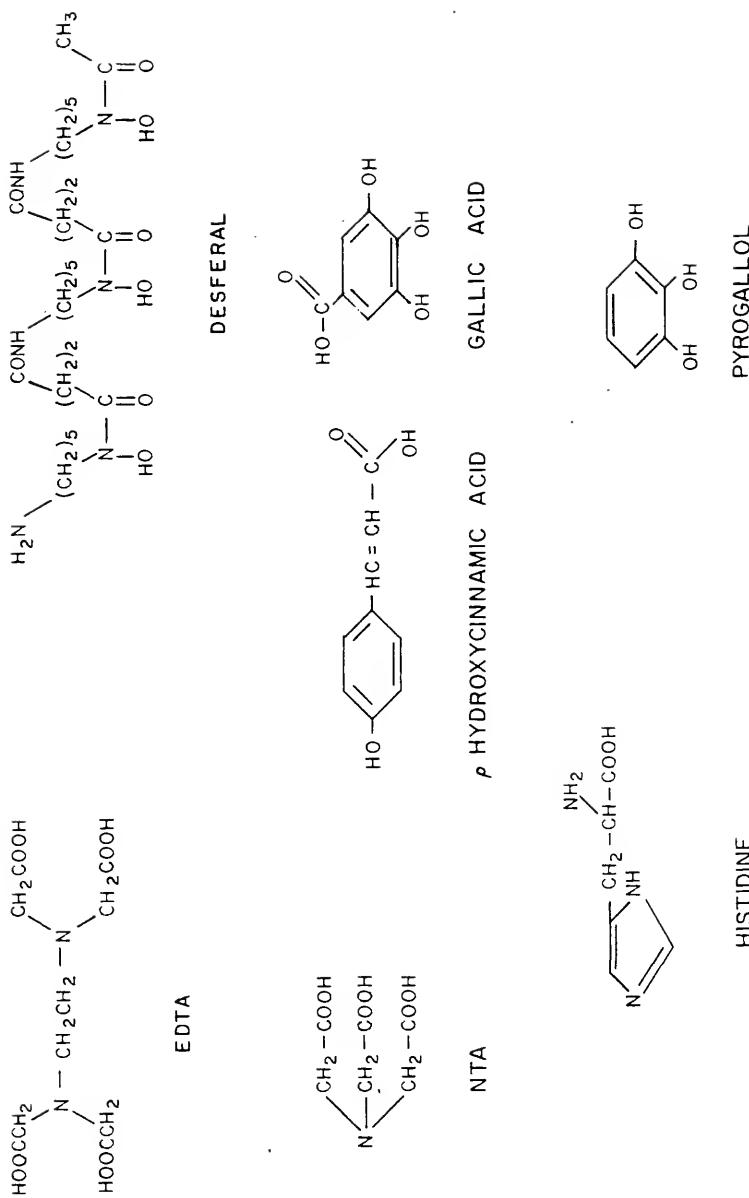


Figure IV-1. Organic compounds used in ASV study.

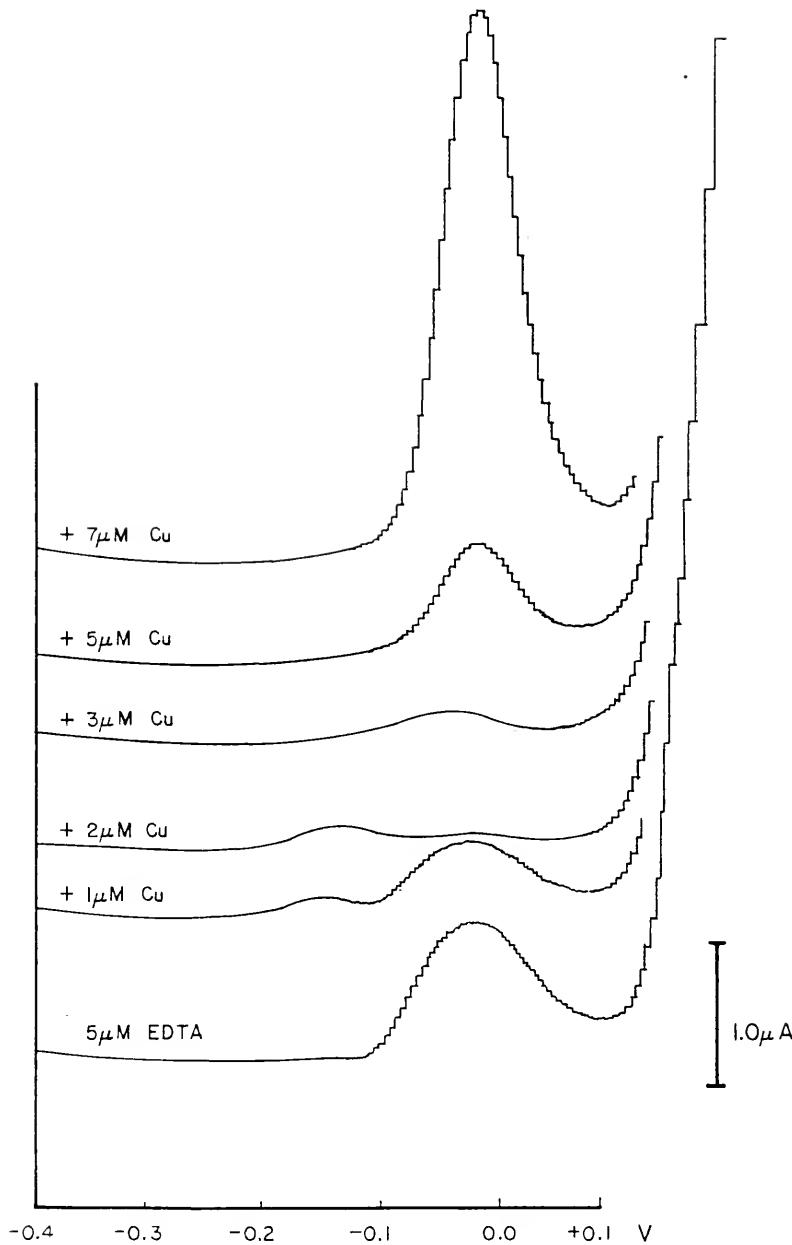


Figure IV-2. Anodic stripping peaks for a solution of EDTA ($5\mu M$) containing copper at varying concentrations.

comparison can be made. For the model compounds, the thermodynamic stability constants from Sillen and Martell (1971) and Ringbom (1963) were converted to conditional values by the method of Ringbom (1963) as follows:

$$\beta' M_m L_n = \frac{[ML]}{[M'][L']} = \frac{\beta M_m L_n}{\alpha_M^m \alpha_L^n}, \quad (4.1)$$

where M' is the metal not complexed by L , L' is the ligand not complexed by M , $\alpha_M = [M']/[M]_{\text{free}}$, and $\alpha_L = [L']/[L]_{\text{free}}$.

For instance, in the case of the titration of EDTA with copper, with hydrogen and calcium as competing cations:

$$\alpha_{\text{EDTA}} = \frac{[Y']}{[Y]_{\text{free}}} = 1 + \frac{[H]}{K_4} + \frac{[H]^2}{K_4 K_3} + \frac{[H]^3}{K_4 K_3 K_2} + \frac{[H]^4}{K_4 K_3 K_2 K_1} + [Ca] \beta_{\text{CaY}}. \quad (4.2)$$

After substituting the appropriate constants ($pK_4 = 10.34$; $pK_3 = 6.24$; $pK_2 = 2.75$; $pK_1 = 2.07$; and $p\beta_{\text{CaY}} = 10.7$) (Ringbom 1963) and concentrations of analysis ($\text{pH} = 7.0$; $p\text{Ca}^{+2} \leq 5.3$), the α_L value was calculated to be $10^{5.4}$. Similarly, the α_{Cu} was calculated to be $10^{0.6}$ for $\text{pH} = 7.0$ and acetate concentration of 0.03 M. Therefore, the thermodynamic stability constant for Cu-EDTA ($\beta = 10^{18.8}$) was converted to the conditional value (β') as follows:

$$\beta'_{\text{Cu-EDTA}} = \frac{\beta_{\text{Cu-EDTA}}}{\alpha_{\text{Cu}} \alpha_{\text{EDTA}}} = \frac{10^{18.8}}{10^{0.6} 10^{5.4}} = 10^{12.8} \quad (4.3)$$

It should be noted that as Cu^{+2} replaced the calcium complexed by EDTA during the titration, the free calcium concentration, $[\text{Ca}^{+2}]$,

increased from $\sim 10^{-6.3}$ M to $\sim 10^{-5.3}$ M. Thus, the conditional stability constant ($\beta' = 10^{12.8}$) represents a lower limit based on the extreme case (i.e., $[Ca^{+2}] = [Ca]_T = 10^{-5.3}$ M).

To confirm the assumption that the Cu-EDTA complex was sufficiently strong and not reducible at the plating potential used for analysis, a pseudopolarogram was constructed using the method of Matson (1968) (Figure IV-3). Each point on the plot represents the amount of reducible copper at a specific plating potential for various solutions. Figure IV-3 shows that a copper solution devoid of chelating agents is plated maximally at -0.3 V but that Cu-EDTA is not reduced 100% until a plating potential of -0.8 V versus SCE is applied. Hence a plating potential of -0.3 V versus SCE should be sufficiently negative to plate ionic and inorganic species of copper without directly reducing the copper complexed to EDTA.

The results of the titration of EDTA with copper (Figure IV-4) produced a conditional stability constant, β' , equal to $10^{7.7}$, which is over five orders of magnitude lower than other published values ($\beta' = 10^{12.8}$) (Ringbom 1963; Sillen and Martell 1971) for similar solution conditions. This discrepancy was apparently caused by dissociation of the complex throughout the initial stages of the titration ($[Cu] \ll [EDTA]$), thus producing a slope (S_L) much greater than expected. If complex dissociation were not occurring, the lower curve in Figure IV-4c (S_L for CuEDTA) would be nearly horizontal with a slope of 1.2×10^{-6} .

Calcium (and other cations) have been reported to cause a shift in the reduction potential of CuEDTA and thus may possibly enhance stripping current due to enhanced rates of dissociation of CuEDTA (Bril and

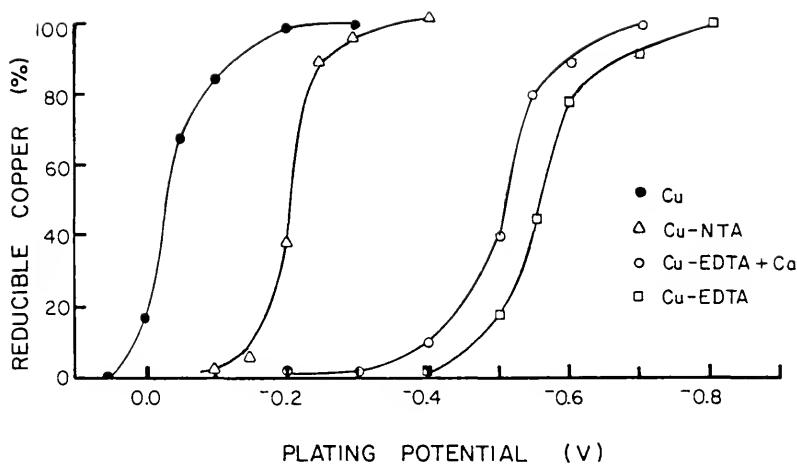


Figure IV-3. DPASV pseudopolarograms of copper ($2 \mu\text{M}$) plus NTA ($5 \mu\text{M}$) or EDTA ($5 \mu\text{M}$) in the presence and absence of calcium ($5 \mu\text{M}$) at pH = 7.0.

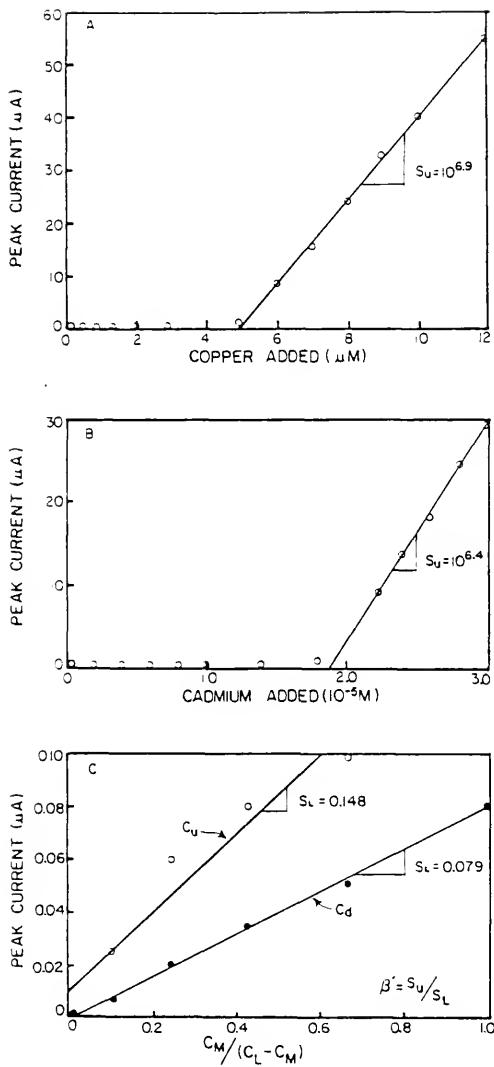


Figure IV-4. DPASV titration curve of (A) $5 \mu\text{M}$ EDTA with copper; (B) $2 \times 10^{-5} \text{ M}$ EDTA with cadmium; and (C) plots of metal (C_M) and ligand (C_L) concentrations for initial additions of copper and cadmium.

Krumholz 1954; Rajput et al. 1978). Further experiments were performed to determine whether the low value of β'_{CuEDTA} that was measured here resulted from the presence of Ca^{+2} . I was able to quantify several points relatively early in an EDTA-copper titration in the complete absence of calcium, since the interfering peak subsided and shifted anodically as copper was added. The resulting peak heights at $C_M/(C_L - C_M)$ values of 0.4 and 0.67 (see Figure IV-4c) did not differ by more than 10% from those in the presence of 5, 10, and 20 μM of calcium, and thus calcium did not enhance the dissociation or reduction of CuEDTA under the conditions of analysis. Additionally, pseudo-polarograms were constructed for CuEDTA solutions in the presence and absence of calcium (Figure IV-3). The half-wave potential for CuEDTA was -0.55 V versus SCE in the absence of calcium, and -0.50 V versus SCE in the presence of 5 μM calcium. This shift of 0.05 V in the reduction potential of CuEDTA was not large enough to cause the observed stripping currents when the plating step was performed at -0.3 V. These results clearly demonstrate that the low value of β'_{CuEDTA} was not caused by the use of Ca^{+2} in the EDTA solutions.

Shuman and Woodward (1973) reported that complex dissociation occurred with CdEDTA and proposed a method to correct for it. Their correction amounted to a 38% increase in β' for CdEDTA at pH = 4.5. EDTA was titrated with cadmium using the differential pulse (DP) mode (Figure IV-3) to compare with the titration reported by Shuman and Woodward (1973), which was done in the DC mode. Experimental conditions were identical to those reported by Shuman and Woodward (1973), except for mode (DP) and EDTA concentration. The higher EDTA concentration used by Shuman and Woodward ($4.45 \text{ to } 17.8 \times 10^{-5} \text{ M}$)

produced nonlinear titration curves in the region beyond the equivalence point when analyzed by DPASV; thus lower levels of EDTA (2–20 μM) were used. The DP mode was selected so that ligand and pH levels typically found in the environment could be investigated, and because under those conditions, DC mode often would require unreasonably long deposition times. After correcting for complex dissociation, I calculated β' for CdEDTA to be $10^{8.5}$, which compares well with the values of Ringbom (1963) ($\beta' = 10^{8.8}$) and Shuman and Woodward (1973) ($\beta' = 10^{8.1}$) for the same solution conditions.

In order to compensate for dissociation of the Cu-EDTA complex using the method of Shuman and Woodward (1973), the correction factor would need to be $10^{5.1}$ to produce an accurate conditional stability constant. Instead, the correction procedure produced negative intercept values, which are physically meaningless and could not be used to correct β' of CuEDTA.

Possibly, partial dissociation of the Cu-EDTA complex during the time scale of measurement by ASV is atypical of the behavior of (thermodynamically) strong copper-organic complexes. Therefore several other organic compounds were investigated. Desferal, a trihydroxamate siderochrome, is a natural microbial growth factor that simulates the functionality and molecular weight (656 daltons) of aquatic and soil-derived humic substances (Wilson and Weber 1977; Reuter and Perdue 1981; Schnitzer 1981). Desferal was recently reported to complex copper with $\beta' = 10^{8.4}$ (McKnight and Morel 1979), based on titrations with a copper ion-selective electrode. Titration of Desferal (10^{-5} M) with copper using DPASV under the same conditions of pH (6.25) and supporting electrolyte (10^{-3} M KNO_3) reported by

McKnight and Morel (1979) revealed that the Cu-Desferal complex was reducible at the mercury electrode (Table IV-1). In solutions of Desferal, copper produced somewhat lower (by 10–50%) and broader peaks than did equal concentrations of free copper ion; however, on an areal basis the peaks were of equal size. The peak potential (E_p) of Cu-Desferal was 100 mV cathodic of E_p for uncomplexed copper, and thus we were unable to plate copper ion without reducing the Cu-Desferal complex. A similar situation was found with Cu-nitrilotriacetic acid (NTA) in that discrete plating of copper ion was not possible (Figure IV-3).

On the other hand, cadmium in the presence of Desferal was only partially reduced during the plating step of ASV. The addition of Desferal to a 2 μM cadmium solution decreased i_p , but even at Desferal concentrations of 1 mM, i_p did not approach 0.0 μA , suggesting that cadmium forms a weak, nonreducible complex with Desferal (O'Shea and Mancy 1976).

Several other organic compounds were studied to determine the extent of copper reduction during analysis by DPASV. Histidine formed a complex with copper that was completely reducible at the mercury electrode (Table IV-1) despite calculations predicting that more than 99% of the copper was complexed with histidine under the conditions of analysis (2×10^{-6} M Cu; 2×10^{-4} M histidine; for $\text{Cu}(\text{HIS})_2$, $\log \beta_2 = 18.1$ and $\log \beta'_2 = 16$). Three other small compounds, p-hydroxycinnamic acid, gallic acid, and pyrogallol, which are simple (monomeric) models of humic material found in natural waters, were analyzed in solutions of 2 μM copper. At ligand concentrations up to 10^{-4} M, the stripping currents were identical to those for

Table VI-1. Effect of model organic compounds on ASV analysis of Cu and Cd.

Model compound	Metal titrant	pH	Metal in presence of ligand*	(Metal ion)	E_p (volts vs. SCE)	Reducible nature of complex**
EDTA	Cu	7.0	- 0.065	(0.0)	Complex predominantly nonreducible	
	Cd	4.5	- 0.60	(- 0.60)	Complex nonreducible	
Desferal	Cu	6.25	- 0.07	(+ 0.025)	Complex reducible	
	Cd	6.25	- 0.58	(- 0.58)	Complex nonreducible, but weak	
Bovine Serum Albumin	Cu	7.0	0.0	(0.0)	Initial complex nonreducible subsequent complexes partially reducible	
	Cu	7.0	0.0	(0.0)	Complex reducible	
Histidine	Cu	7.0	0.0	(0.0)	Complex reducible	
p-hydroxycinnamic acid	Cu	7.0	0.0	(0.0)	Complex reducible	
Gallic acid	Cu	7.0	0.0	(0.0)	Complex reducible	
Pyrogallol	Cu	7.0	0.0	(0.0)	Complex reducible	

*Most negative value of E_p obtained during titration.
 **For plating potentials θ_f -0.3 V (Cu) and -0.8 V (Cd).

uncomplexed copper. No stability constants for these three compounds are available in the literature to calculate the extent of copper complexation, but some complexation seems likely at these concentrations of ligand.

A large molecular weight organic compound, bovine serum albumin (BSA), was used as a model of the macromolecular dissolved organic nitrogen in natural waters and was titrated with copper. A very strong, nonreducible complex was observed initially, followed by weaker binding. No discernable break was found in the titration curve, thus precluding an accurate determination of β' . Bovine serum albumin has one very strong binding site and at least 11 other weak binding sites, some of which act more like ion exchange sites than binding sites (Klotz and Curme 1948). The nature of BSA exemplifies the problems involved in analyzing a heterogeneous mixture of organic compounds, such as those found in natural waters. For example, with naturally occurring organic matter others (Mantoura and Riley 1975; Guy and Chakrabarti 1976; Bresnahan et al. 1978; Giesy 1978) have reported at least two distinct classes of binding sites (based on Scatchard plots). The binding sites typically varied from strong to weak as metal concentration increased. The ASV titration procedure does not identify the various classes of available binding sites, and calculation of β' from such titrations is based on the first few copper additions ($C_M \ll C_L$ [Shuman and Woodward 1977]), which probably represent only the stronger binding sites, not the average. Thus for a mixture of compounds with various binding sites, the β' probably would be overestimated by the ASV procedure, the convoluting problems of complex dissociation and direct complex reduction aside.

It is concluded that, as currently practiced, the complexometric titration with copper, using ASV with a stationary mercury electrode, is not an accurate method either for determining complexing capacities (C_L) of natural water samples or for estimating stability constants (β') of copper with natural organic matter. The titrimetric ASV procedure has not been validated with any of the pure compounds investigated using copper as the titrant. Correct C_L values could not be obtained for a wide variety of organic compounds. Although titrations of EDTA gave the correct value of C_L , the calculated β' was over five orders of magnitude in error.

Inaccuracies in determining complexing capacities by this method arise from at least two sources, namely:

1. Rapidly dissociating or directly-reducible metal-organic complex under conditions that predict an "inert" complex.
2. Lack of plating potentials for some environmentally significant metal-organic complexes that are sufficiently separated from the reduction of copper ion.

For a variety of organic ligands that form thermodynamically strong complexes with copper, at most slight decreases in i_p were found when the plating step was performed at -0.3 V, which was the least negative potential that could be used to plate copper ion under my experimental conditions. The ASV copper-titration method thus apparently does not measure C_L for: (1) most inorganic ligands (Ernst et al. 1975; Shuman and Woodward 1977); (2) many small organic ligands that form moderately strong copper complexes (e.g., NTA, histidine); and (3) some large, naturally occurring organic species that also have relatively high β'

values with copper (e.g., Desferal). However, when using the method with natural water samples, one does obtain a titration curve with an "apparent" equivalence point. The work with model compounds (above) does not rule out the possibility that naturally occurring organics (e.g., proteins, humics) form non-reducible complexes. Further evaluation of the ASV method with humics from natural waters is presented in the following section, and the work described there does provide an explanation for the "positive" results obtained with natural water samples.

It has been demonstrated that the above problems occur frequently with copper as a titrant, but the possibility that other metal ions may be more suitable titrants for complexing capacities is not precluded by this work. Cadmium apparently forms more ASV-nonlabile complexes with organic ligands than does copper, probably due to the electron configuration of the d-orbitals, which are filled for Cd(II) (d^{10}) but only partially filled for Cu(II) (d^9). However, cadmium forms complexes with organic ligands that are much less stable (thermodynamically) than does copper, and the complexing capacities thus obtained may not be comparable to the true complexing capacities of natural organic compounds with copper. Hanck and Dillard (1977b) used indium (III) as a titrant for complexing capacities because In(III) typically forms stronger complexes than copper. However, the same problems of interpreting such complexing capacities relative to binding capacities for other metal ions still remain, and the environmental significance of indium itself is rather small.

The same deficiencies stated above for complexing capacities apply to the estimation of conditional stability constants. In addition, the

problem observed with BSA may be common to macromolecular organic matter in natural waters; i.e., the average conditional stability constant determined by this method is based on the first few additions of metal, which probably does not accurately represent the average value for all ligand sites on the macromolecule. Although the latter was observed only with copper, it should occur with other metal titrants as well.

B. Comparison of Five Methods to Determine C_L and β'

1. ASV Titration

Although I could not verify the validity of the ASV method using model compounds, it is possible that the method gives accurate results for complexation by natural water macromolecules such as humics. Many researchers have used it for this purpose, assuming it produces valid results. Because of its widespread use, the method was included in the comparative study in spite of the reservations derived from work in the previous section. If the ASV method produced results that were comparable to the other four methods, then perhaps the method works for natural water organic ligands despite the discrepancies obtained with model compounds. However, results inconsistent with the other four methods would cast further doubt on the validity of the ASV method.

The titrations of Waldo and Basin swamps' samples with copper at pH 6.25 and 0.1 M KNO₃ resulted in the curves illustrated in Figure IV-5. The data were treated in two ways, first by the method of Shuman and Woodward (1977) to determine C_L and β' and additionally by the

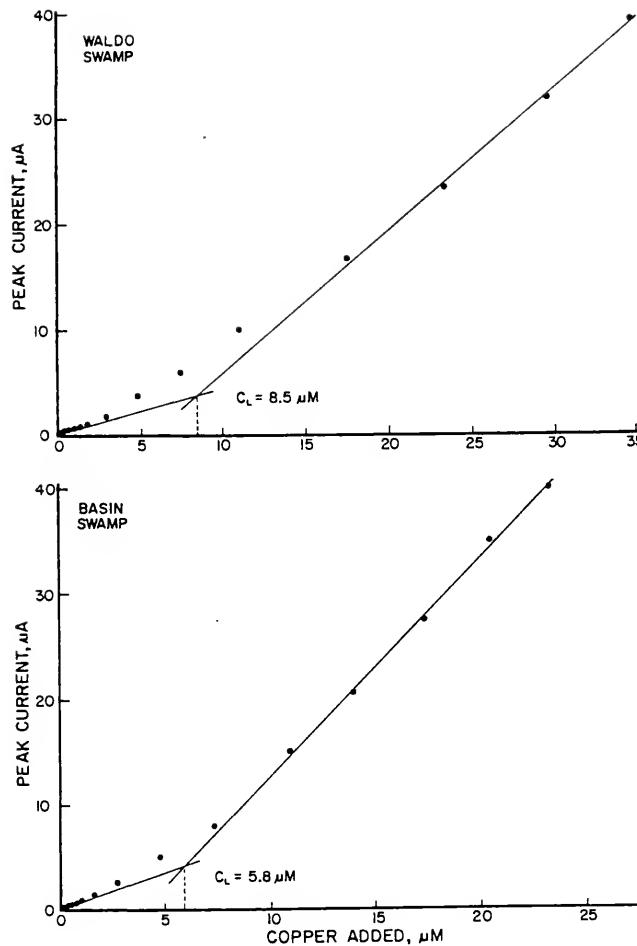


Figure IV-5. ASV titration curve of Waldo Swamp and Basin Swamp samples with copper.

method of Scatchard (1949). The former method produced C_L values of 8.5 and 5.8 μM (Figure IV-5) and β' values of $2.5 \cdot 10^5$ and $5.2 \cdot 10^5$ for copper with Waldo and Basin swamps' waters, respectively (Table IV-2).

Because the procedure described by Shuman and Woodward (1977) applies only to the ASV technique, the data were treated by Scatchard analysis so that a direct comparison with other procedures could be made. The data in Figure IV-5 were converted to concentrations of copper bound, (Cu_B), and not bound, (Cu_f), to the soluble organic matter of interest. The slope of the curve after the equivalence point, S_u , was assumed to represent the behavior of uncomplexed metal, and therefore i_p values prior to the equivalence point were divided by S_u to determine concentrations of uncomplexed copper. The concentrations of complexed copper were calculated from the difference between the concentrations of total and uncomplexed copper. Subsequently, values of \bar{V} (where $\bar{V} = [\text{Cu}_B]/[\text{L}_t]$), and $\bar{V}/(\text{Cu}_f)$ were calculated and plotted (Figure IV-6).

The Scatchard plots were segmented into three sections so that a comparison could be made among the five procedures. The slope of the most nearly linear line in each section (determined by linear regression) is the reported value of β' . Scatchard analysis of both Waldo and Basin swamps' samples produced values in the lower two ranges of \bar{V} only. For the Waldo Swamp sample, similar β' values were obtained for each range ($1.8 \cdot 10^5$ and $2.3 \cdot 10^5$; Table IV-2). These values are similar to β' determined using the Shuman and Woodward (1977) method of calculation ($\beta' = 2.5 \cdot 10^5$). Scatchard analysis of the Basin Swamp sample resulted in two different slopes, and β' values

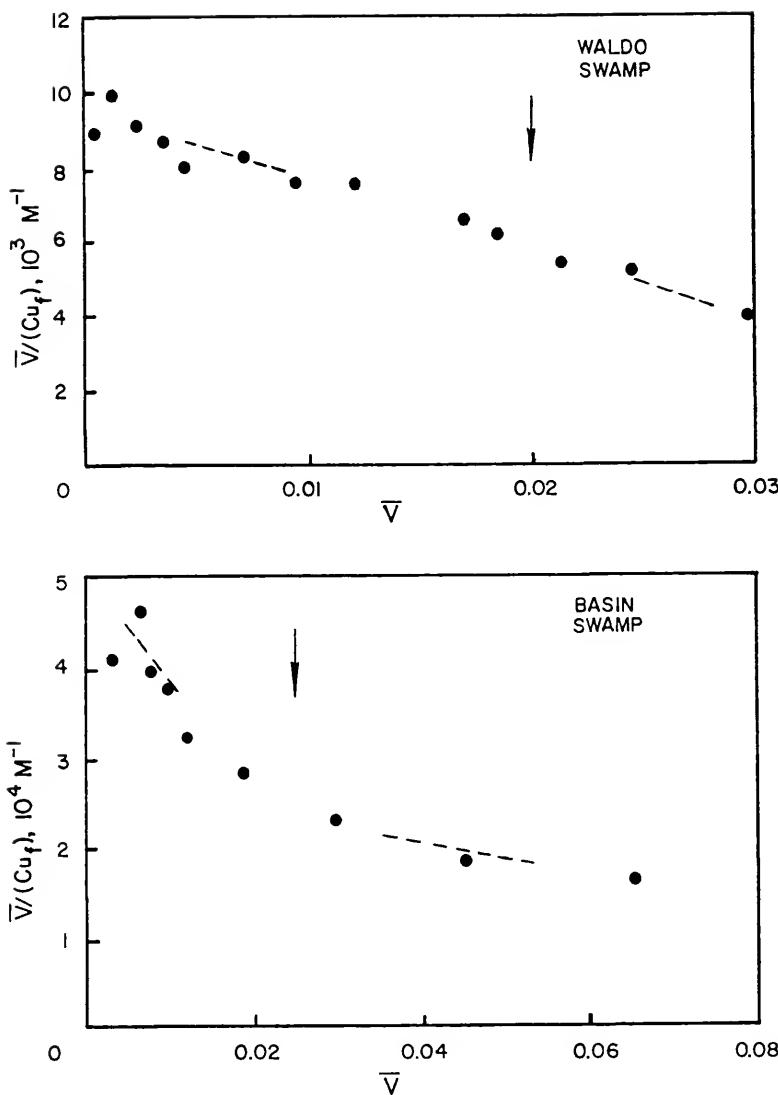


Figure IV-6. Scatchard plots for copper titrations of water samples using ASV technique. Arrows delimit linear segments (dashed lines).

Table IV-2. Copper binding capacities and conditional stability constants for water samples and model compounds using ASV.

	Copper Binding Capacity	\bar{V} Range	Molarity of V Range	β'	$\log \beta'$
Waldo Swamp	$8.5 \cdot 10^{-6}$ M	0-0.02	$5.2 \cdot 10^{-6}$	$1.8 \cdot 10^5$	5.25
		0.021-0.2	$4.7 \cdot 10^{-6}$	$2.3 \cdot 10^5$	5.36
		0.21-0.8	---	---	---
Basin Swamp	$5.8 \cdot 10^{-6}$ M	0-0.025	$2.2 \cdot 10^{-6}$	$1.1 \cdot 10^6$	6.04
		0.026-0.125	$5.0 \cdot 10^{-6}$	$2.0 \cdot 10^5$	5.30
		0.126-0.32	---	---	---

were $1.1 \cdot 10^6$ and $2.0 \cdot 10^5$ for the two ranges (Table IV-2). By comparison, the β' value determined by the Shuman and Woodward method was $5.2 \cdot 10^5$. It should be mentioned that the Shuman-Woodward method of calculation gives β' values that are based on the early part of the titration, and hence β' values calculated thusly should be related to the initial value determined in a Scatchard plot.

The addition of cadmium and zinc to Waldo Swamp and Basin Swamp waters resulted in completely reducible species of each metal. Therefore, no values of C_L or β' could be determined for cadmium and zinc by the ASV-titrimetric procedure.

The discussion in subsequent sections reveals a significant discrepancy in the Scatchard-derived values of β' and C_L for copper determined with data from the ASV technique and those values determined using data from the other methods. Consequently, reasons for this discrepancy were sought. An underlying assumption stated for the ASV technique is that the metal-ligand complex be nonlabile (i.e., not reduced at the mercury electrode) (Shuman and Woodward 1973, 1977). As shown in the previous section, complexes of copper with a variety of

simple model organic ligands, as well as those with some relatively large and complicated molecules (e.g., Desferal), are ASV-labile. The question that remains to be addressed is whether copper complexes with humic/fulvic acids are ASV-labile or nonlabile. In that regard, Buffle et al. (1976) and Greter et al. (1979) concluded that lead complexes with humic materials are ASV-labile. Buffle and coworkers suggested that the diffusion coefficient for the large lead-humic complex molecules is smaller than that for ionic lead. The slower diffusion rate for the complexed metal in the zone near the electrode surface thus leads to less reduction of metal and a decrease in peak current, i_p . The relationship between peak current and diffusion coefficients is given for a stationary electrode by a modified version of the Ilkovic equation, which states that current varies with the square root of the diffusion coefficient, D:

$$i_p = k n^{3/2} A D^{1/2} C v^{1/2} \quad (4.4)$$

where k is a constant, n is the number of equivalents of electrons involved per mole of species oxidized or reduced, A is the electrode area in cm^2 , C is the concentration of analyte in moles/L, and v is the scan rate in V/s (Nicholson and Shain 1964). Hence a typical complexometric titration curve (e.g., Figure IV-5) would be obtained using ASV even if the complexed metal were reducible. If such is the case for naturally occurring organic matter with copper or cadmium, then the present interpretation of the ASV complexometric titration is erroneous. Therefore, the possibility was examined that humic complexes with metals other than lead are reducible, but at a rate different than for ionic metal.

Cyclic voltammetry was performed to investigate the reduction and oxidation of copper and cadmium in the presence and absence of organic matter. Cyclic voltammetry involves rapid scanning (50–100 mV/s) from an initial potential that is anodic (i.e., positive) compared to the reduction potential of the metal of interest, to a potential that is more cathodic than the reduction potential of the metal. This "forward" scan is followed immediately by a potential scan in the reverse direction to the original potential. The forward scan produces a reduction of metal and a current deficiency (negative peak); the reverse scan causes oxidation and a release of electrons. A cyclic voltammogram for 10^{-5} M copper and background electrolytes (0.01 M KNO_3 ; pH 6.25) is shown in Figure IV-7. The reduction peak is at +0.01 V versus SCE and the oxidation peak is at +0.04 V versus SCE. The 30 mV difference is expected for a reversible process involving two electrons (Nicholson and Shain 1964).

In the presence of organic matter (Waldo Swamp water), the reduction peak of copper is shifted to -0.11 V versus SCE, and a decrease in current is observed. This peak is likely due to the direct reduction of complexed copper, and the low and rounded nature of the reduction peak indicates that the copper complex diffused at a lower rate than Cu^{+2} (Greter et al. 1979). The broader nature of the peak suggests also that the reduction process was irreversible (Nicholson and Shain 1964). The copper oxidation peak is a shoulder on the rising mercury oxidation peak, which is shifted cathodically due to the presence of organic matter (Figure IV-7). The magnitude of the oxidation peak is difficult to quantify due to the sloping baseline. Most likely the cathodic shift in the oxidation of mercury is caused by complexation of

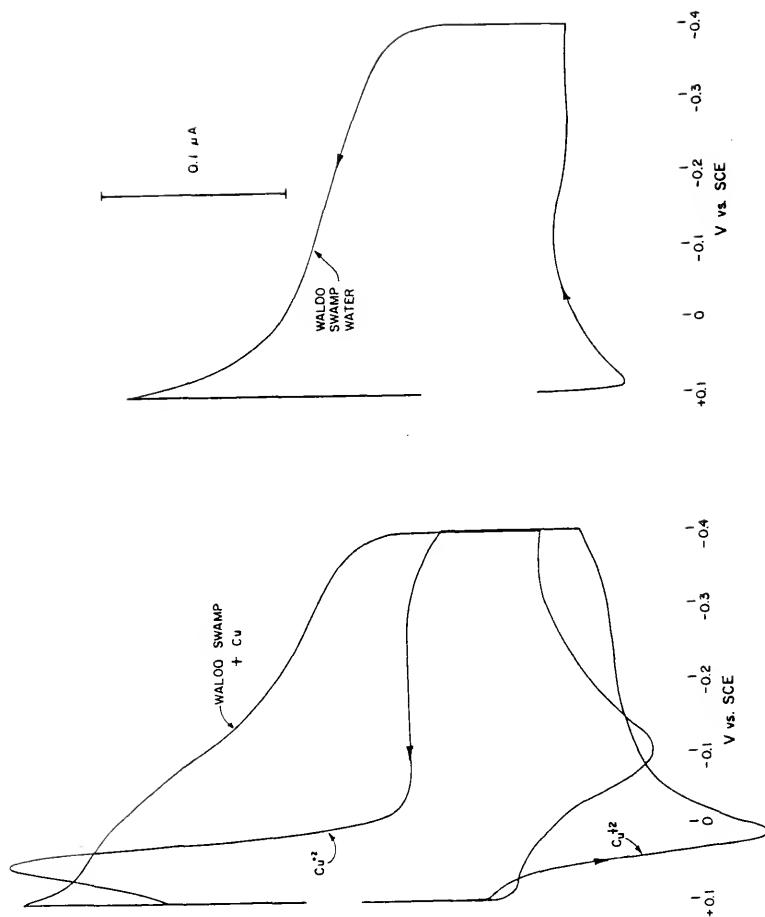


Figure IV-7. Cyclic voltammograms of 10^{-5} copper, Waldo Swamp water plus 10^{-5} copper, and Waldo Swamp water alone.

mercury by soluble organic compounds. As copper titrant is added, less organic matter is able to complex with mercury, and the baseline subsides. Therefore, due to the varying nature of the oxidation potential of mercury (and hence the changing baseline), the oxidation of copper in the presence of strongly complexing organics cannot be quantified accurately when using a mercury electrode.

Nevertheless, the copper-organic complex examined here appears to be readily reducible at a potential 120 mV cathodic of the reduction potential of Cu^{+2} . This difference in potentials is not great enough to allow plating of Cu^{+2} without reducing the complexed metal. Consequently, the concentration of free Cu^{+2} ion cannot be measured accurately in this case. A second experiment (Figure IV-8) indicated that adsorption of organic matter to the electrode was occurring, because the magnitude of the reduction peak increased with increased time of waiting after forming the mercury drop, both with and without an applied potential. No such effect was observed for solutions containing only copper or only organic matter. Buffle et al. (1976) observed the same increase in reduction current for lead with increasing waiting times, but they also reported a cathodic shift in reduction potential. No shift in reduction potential with varying waiting times was observed for copper here.

As noted earlier in this section, the addition of cadmium and zinc to Waldo and Basin swamps' samples resulted in completely reducible species of each metal. The electrochemical behavior of these reducible cadmium species was investigated further. Cyclic voltammograms of cadmium ($5 \cdot 10^{-6}$ M) in background electrolytes only, and cadmium in the Waldo Swamp water (pH 6.25; 0.01 M KNO_3) are presented in Figure

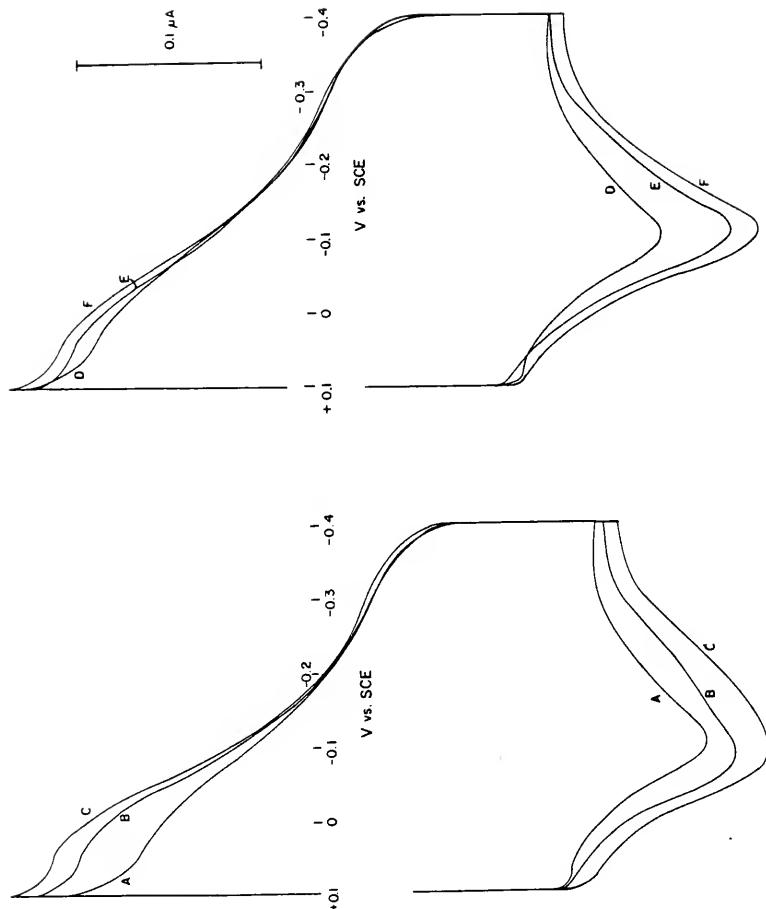


Figure IV-8. Cyclic voltammograms of 10^{-5} copper in Waldo Swamp water at waiting times (without applied potential) of 3 s (A), 60 s (B), and 120 s (C), and with an applied potential of 3 s (D), 60 s (E), and 120 s (F).

IV-9. The cyclic voltammograms of cadmium in background electrolytes shows a reduction peak at -0.585 V versus SCE and an oxidation peak at -0.555 V versus SCE indicating the expected reversible behavior. For cadmium in the Waldo Swamp sample, both the reduction and oxidation peaks are shifted 30 mV in the cathodic direction. However, the reduction current (peak) is greater for cadmium in the Waldo Swamp water than in the electrolytes alone. This increase in reduction current for cadmium in the presence of organics appears to be caused by adsorption of organically complexed cadmium to the surface of the electrode. In another experiment (scans not shown), the reduction current increased with increasing waiting time after drop formation (both with and without an applied potential) in a manner similar to that observed with copper and the Waldo Swamp water. The results obtained using the continuous ultrafiltration technique (section IV-B-4) indicate that a cadmium-organic matter complex was present in the Waldo Swamp water. Cyclic voltammograms show that the cadmium complex was ASV-labile with reduction occurring at a potential 30 mV cathodic of Cd⁺².

In addition to the surface water samples, three homopolyamino acids—polyarginine, polyalanine, and polyaspartic acid—were examined by ASV. These polymers range in molecular weight from 3900 to 13,900 daltons and were expected to simulate the metal binding character of natural organic matter. However, the addition of cadmium, copper, and zinc to each of the polyamino acids all resulted in reducible species of metal and consequently β' and C_L could not be determined from ASV titrations. Using other metal-speciation techniques that are discussed subsequently, polyaspartic acid was found to complex copper, cadmium,

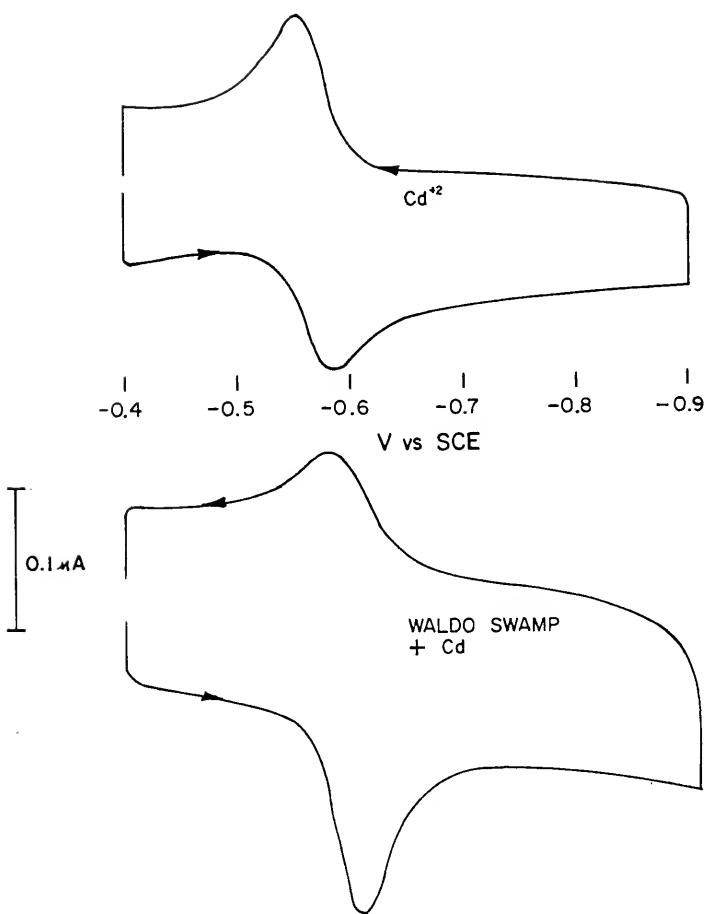


Figure IV-9. Cyclic voltammograms of Cd ($5 \mu\text{M}$) in background electrolytes and in Waldo Swamp water.

and zinc. Therefore, heavy metal complexation by polyaspartic acid was examined by cyclic voltammetry.

The scans for copper (10^{-5}) and polyaspartic acid ($1.8 \cdot 10^{-5}$ M) produced a reduction peak that was lower, broader, and 120 mV more cathodic than the reduction peak for a solution of Cu^{+2} containing no organics (Figure IV-10). However, the oxidation peak for copper with polyaspartic acid was only 50 mV cathodic of the oxidation of Cu^{+2} . For a reversible two-electron process, the difference between peak potential for the reduction and oxidation processes should be 30 mV using cyclic voltammetry (Nicholson and Shain 1964), whereas the difference observed here was 100 mV (Figure IV-10). Consequently the reversibility of the copper-polyaspartic complex is in question. The decrease in peak current for the polyaspartic acid solution indicates that the reduction process is slower for the copper-polyaspartic acid complex than for Cu^{+2} , and therefore the diffusion rate is slower for the complexed copper than for Cu^{+2} .

The adsorption phenomenon observed for the Waldo Swamp sample was not detected for polyaspartic acid. No change in potential or current occurred for various waiting times after drop formation. This result is in agreement with the visual observation that discarded drops of mercury remained discrete in the bottom of the cell for the Waldo Swamp sample (due to a change in surface tension), whereas the mercury drops coalesced in solutions of polyaspartic acid and solutions containing only inorganic species.

Further evidence of a reducible copper-polyaspartic acid complex was obtained by doubling the polyaspartic acid concentration of a solution containing $1.8 \cdot 10^{-5}$ M polyaspartic acid and 10^{-5} M copper. The

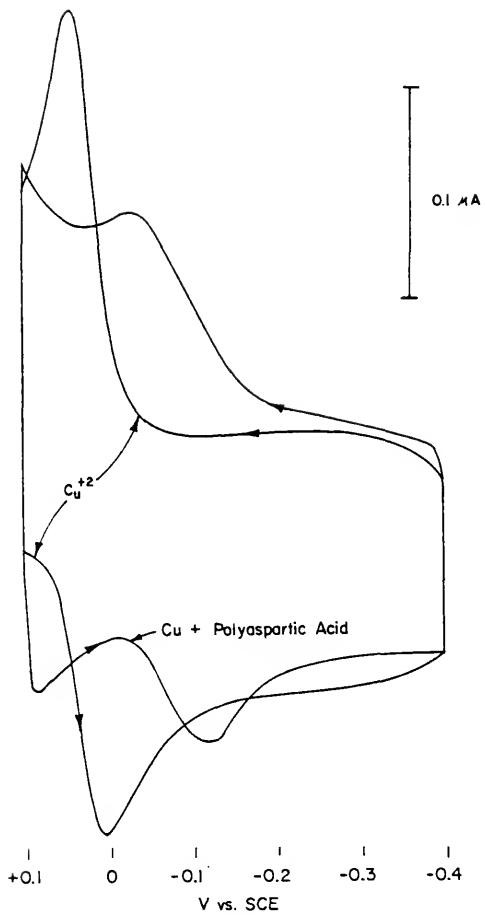


Figure IV-10. Cyclic voltammograms of copper (10^{-5} M) in background electrolytes and in polyaspartic acid ($1.8 \cdot 10^{-5}$ and $3.6 \cdot 10^{-5}$ M).

cyclic voltammograms before and after increasing the polyaspartic acid concentration were identical, which suggests that the reduction peak was due to the direct reduction of a copper-polyaspartic acid complex. Assuming nearly complete complexation of copper, increases in polyaspartic acid would not affect appreciably the concentration of the copper complex. However, if the reduction peak were due to the reduction of Cu^{+2} , then an increase in polymer concentration would decrease the peak further. Therefore, these results indicate that the copper-polyamino acid complex exists in appreciable concentrations and that the complex is reducible at a potential near that for Cu^{+2} . The same conclusions can be applied to the Waldo Swamp sample. The shapes of the cyclic voltammograms for both the Waldo Swamp water and the polyaspartic acid solution with copper are remarkably similar (c.f., Figure IV-7).

Cyclic voltammograms of cadmium ($5 \cdot 10^{-6}$ M) in a solution of polyaspartic acid ($1.8 \cdot 10^{-5}$ M) resulted in oxidation and reduction potentials that were each 30 mV cathodic of the potentials for Cd^{+2} . In addition, the reduction and oxidation currents were slightly lower for cadmium in the solutions of the polymer than in inorganic solutions of cadmium. Scans of solutions containing polyaspartic acid and cadmium did not vary with waiting time, and therefore adsorption phenomenon was not observed.

The results presented here indicate that copper and cadmium complexed with the organic matter in the Waldo Swamp sample and the polyaspartic acid, but that the metal-organic complexes were reducible at a potential near that for ionic metal. As a result, the concentration of ionic metal could not be determined accurately in the presence of the

organics investigated. The underlying assumption for the ASV-titrimetric procedure that states that the metal-organic complexes are not reduced at the mercury electrode thus has not been supported either for model compounds or for natural water organics.

2. Ion-Selective Electrode Titration

The results of the copper titration of Waldo and Basin swamps' samples using a copper ion-selective electrode are presented in Figure IV-11. For equivalent additions of copper, the Waldo Swamp sample produced lower levels of Cu^{+2} than did the Basin Swamp sample, indicating that the former sample complexed copper to a greater extent. The binding capacity was difficult to assess using the plot in Figure IV-11, but a plot of the concentrations of bound copper, Cu_b , versus concentrations of total added copper, Cu_t , shown in Figure IV-12 allowed values of C_L to be determined. The resulting copper binding capacities, C_L , were $7.9 \cdot 10^{-5} M$ and $1.4 \cdot 10^{-5} M$ for Waldo and Basin swamps' samples, respectively.

The stability of the copper-organic complexes was evaluated by Scatchard analysis (Figure IV-13). Each curve was divided into three sections, and the slope of the most nearly linear line through the points in each section is the reported value of β' . For the Waldo Swamp sample, β' values were $1.3 \cdot 10^8$, $5.3 \cdot 10^6$, and $2.2 \cdot 10^5$ for \bar{V} ranges of 0–0.02, 0.021–0.2, and 0.21–0.8, respectively (Table IV-3). Titration of the Basin Swamp sample produced conditional stability constants that were slightly lower than those for the Waldo Swamp sample. The β' values for Basin Swamp were $6.6 \cdot 10^7$, $7.1 \cdot 10^6$, and $8.0 \cdot 10^4$ for \bar{V} ranges of 0–0.025, 0.026–0.125, and 0.125–0.32, respectively. The \bar{V} -intercept

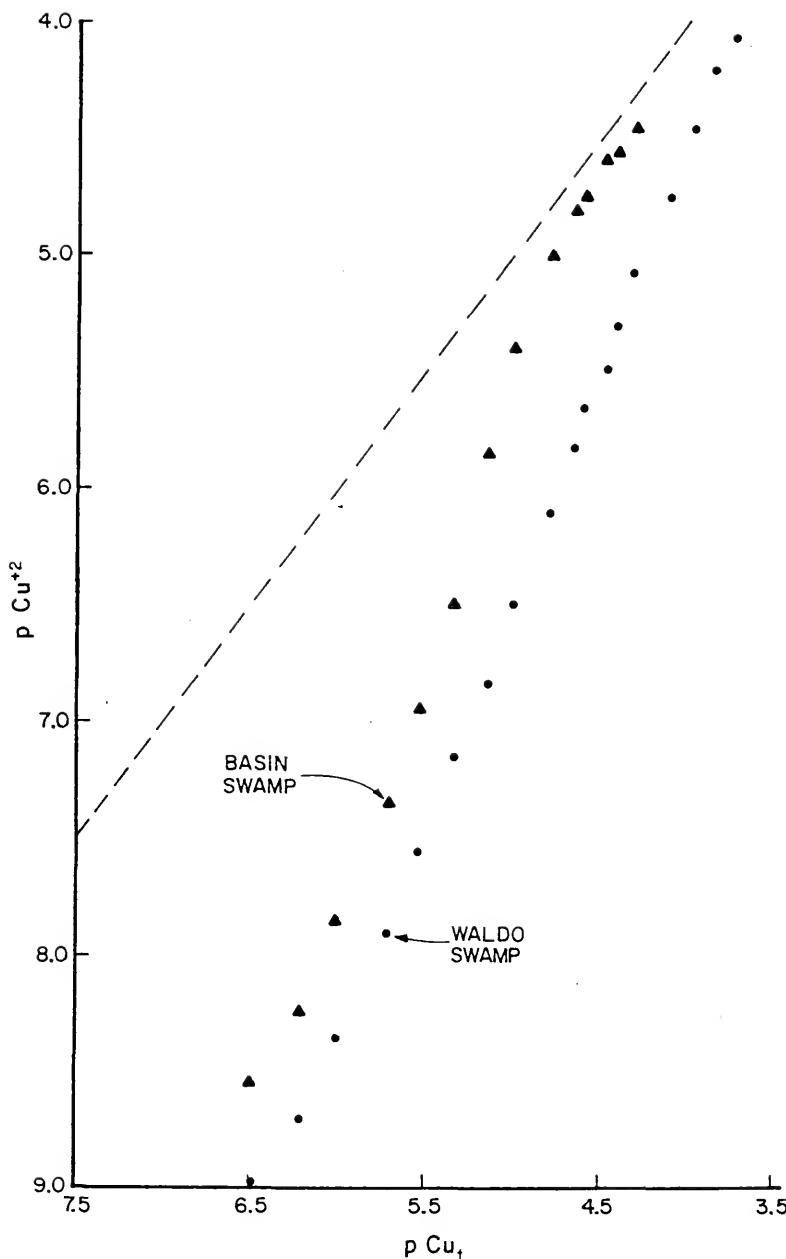


Figure IV-11. Copper titrations curves of Waldo and Basin swamp water samples, and background (dashed line) using ISE method.

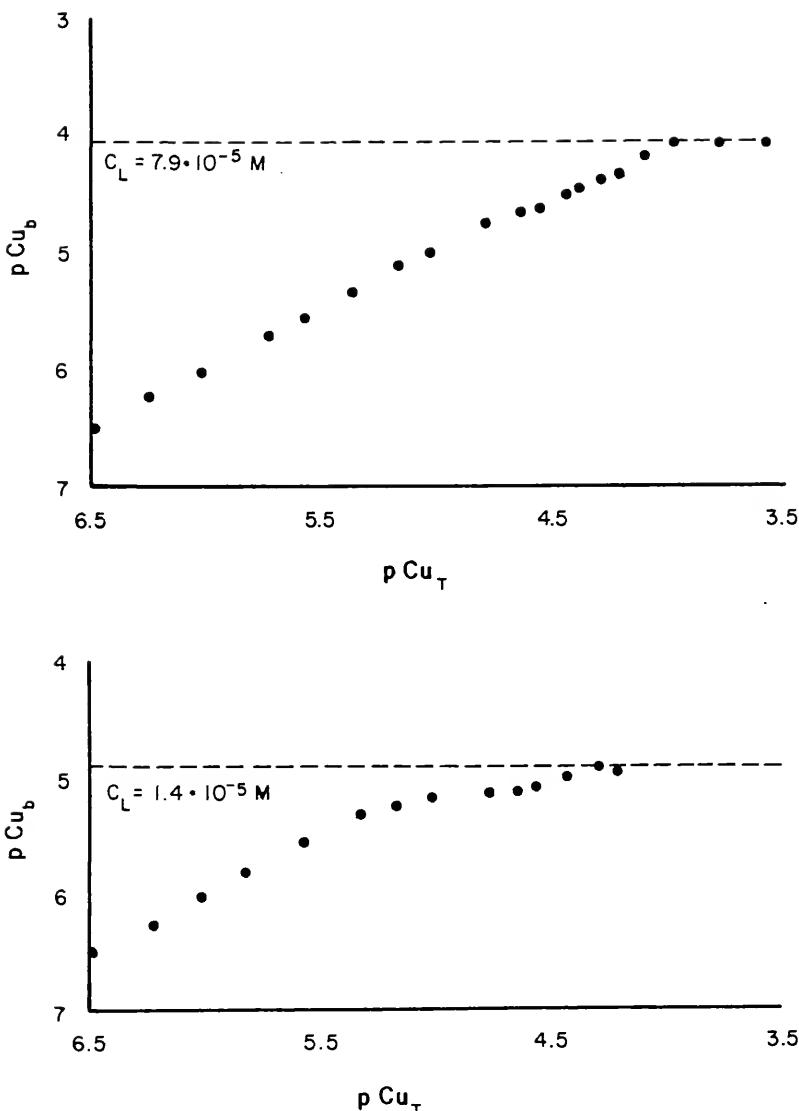


Figure IV-12. Binding curves for copper titrations of Waldo Swamp (upper) and Basin Swamp (lower) water samples using ISE method.

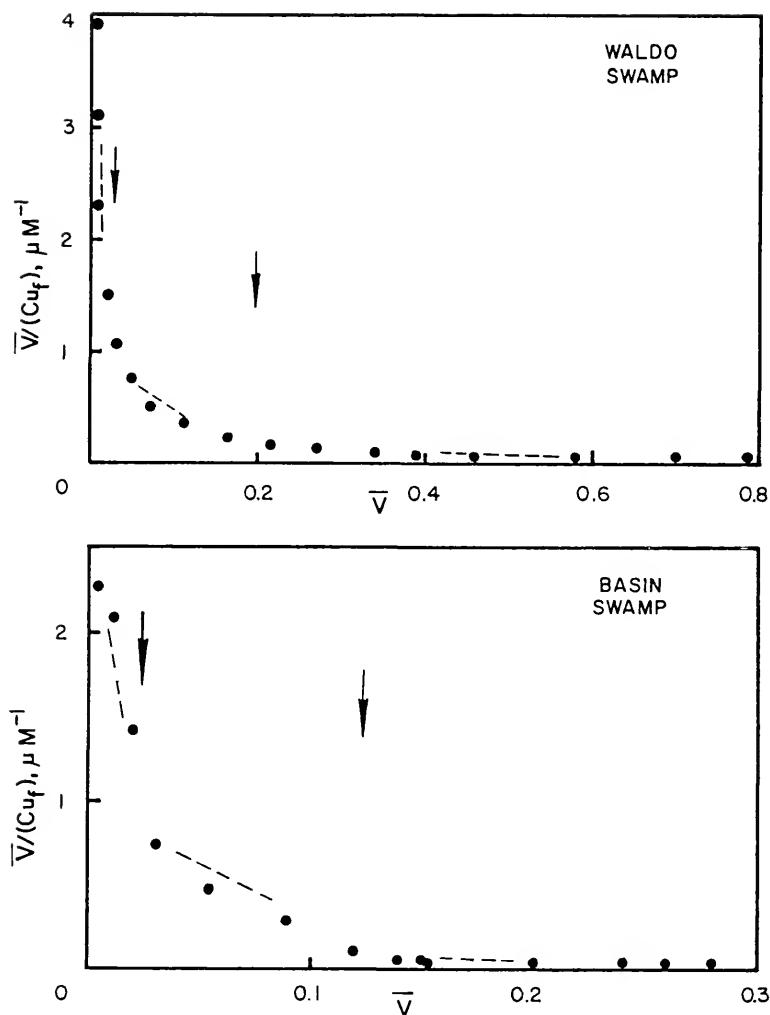


Figure IV-13. Scatchard plots for copper titrations of water samples using ISE method. Arrows delimit linear segments (dashed lines).

Table IV-3. Copper binding capacities and conditional stability constants for water samples and model compounds using ISE.

	Copper Binding Capacity	\bar{V} Range	Molarity of V Range	β'	$\log \beta'$
Waldo Swamp	$7.9 \cdot 10^{-5}$ M	0-0.02	$3 \cdot 10^{-6}$	$1.3 \cdot 10^8$	8.11
		0.021-0.2	$1.6 \cdot 10^{-5}$	$5.3 \cdot 10^6$	6.72
		0.21-0.8	$6 \cdot 10^{-5}$	$2.2 \cdot 10^5$	5.34
Basin Swamp	$1.4 \cdot 10^{-5}$ M	0-0.025	$2.1 \cdot 10^{-6}$	$6.6 \cdot 10^7$	7.82
		0.026-0.125	$4.4 \cdot 10^{-6}$	$7.1 \cdot 10^6$	6.85
		0.126-0.32	$1.0 \cdot 10^{-5}$	$1.8 \cdot 10^5$	5.26
Polyaspartic Acid ($3.7 \cdot 10^{-5}$ M)	$5 \cdot 10^{-4}$ M	0-0.25	$1.2 \cdot 10^{-5}$	$3 \cdot 10^8$	8.50
		0.26-4	$1.36 \cdot 10^{-4}$	$1.1 \cdot 10^7$	7.04
		4.1-14.7	$2.58 \cdot 10^{-4}$	$2.7 \cdot 10^5$	5.44
Polyarginine ($2.9 \cdot 10^{-4}$ M)	$< 3 \cdot 10^{-7}$ M	---	---	---	---
Polyalanine ($5 \cdot 10^{-5}$ M)	$< 3 \cdot 10^{-7}$ M	---	---	---	---

values of each linear segment can be interpreted as the approximate number of binding sites per molecule of ligand. Hence, for the Waldo Swamp water with a ligand concentration of 10^{-4} M, there were $3 \cdot 10^{-6}$ M of sites with moderately strong stability, $1.6 \cdot 10^{-5}$ M of sites with intermediate stability, and $6 \cdot 10^{-5}$ of sites with weak stability (Table IV-3). Similarly, the Basin Swamp sample contained $2.1 \cdot 10^{-6}$ M of sites with moderately strong stability, $4.4 \cdot 10^{-6}$ M of sites with intermediate stability, and $1.0 \cdot 10^{-5}$ M of sites with relatively weak stability (Table IV-3).

The three model compounds analyzed by the ISE technique were polyarginine, polyalanine, and polyaspartic acid. Only polyaspartic acid was found to complex copper. One obvious difference between polyaspartic acid and the other two polymers is that the monomeric component of the former contains a carboxyl group in addition to the amino acid moiety, whereas the latter are composed of monomers with neutral or basic side chains. Perhaps the differences in functionality were responsible for the observed metal binding behavior, but the cause could be due to differences in polymeric structure, about which information was not available.

The copper binding capacity of a $3.7 \cdot 10^{-5}$ M solution of polyaspartic acid was found to be $5 \cdot 10^{-4}$ M (Table IV-3), which indicates that each molecule of ligand complexed an average of 13.5 Cu^{+2} ions. The stability of the copper-polyaspartic acid complexes was analyzed by segmenting the Scatchard plot in a manner similar to that described for the surface water samples (Figure IV-14). The β' values for the \bar{V} ranges—0–0.25, 0.26–4, and 4.1–14.7—are $3 \cdot 10^8$, $1.1 \cdot 10^7$,

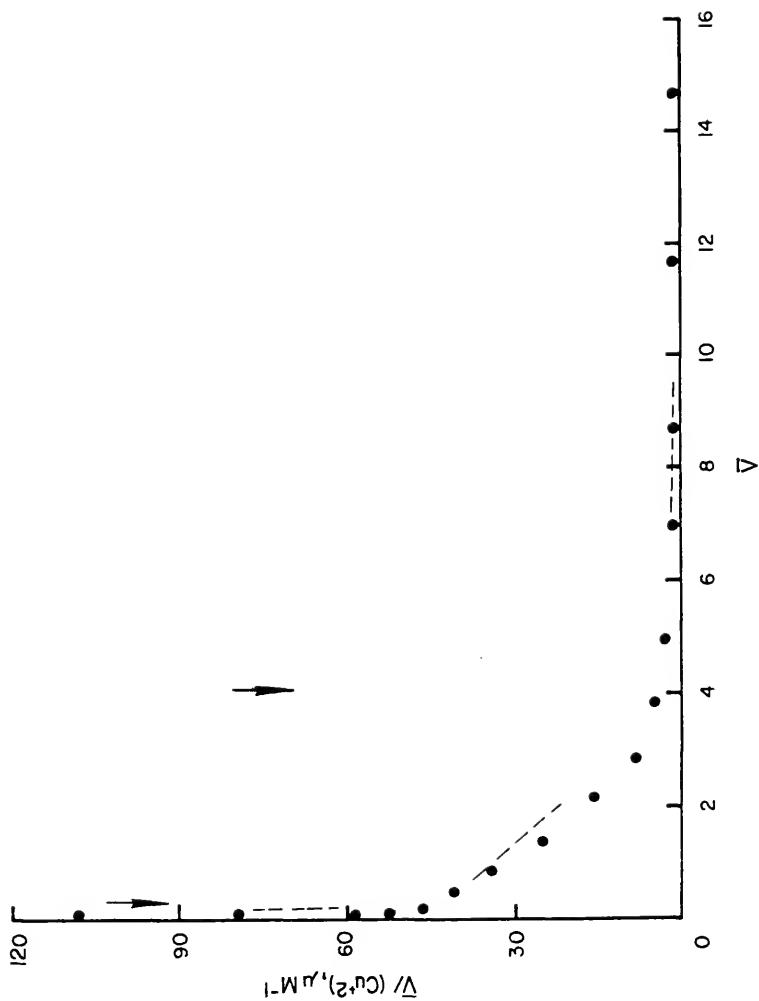


Figure IV-14. Scatchard plot for the titration of polyaspartic acid with copper using ISE method. Arrows delimit linear segments (dashed lines).

and $2.7 \cdot 10^5$, respectively (Table IV-3). As expected, the stability of the copper complexes decreases with increasing levels of copper. The molarity of the first range of binding sites ($\bar{V} = 0\text{--}0.25$) is $1.2 \cdot 10^5$, which corresponds to 0.32 moles of copper bound (at $\beta' = 3 \cdot 10^8$) per mole of polyaspartic acid. This fractional value suggests that either multidentate binding occurred or the polymer was heterogenous. The molarities of the two subsequent \bar{V} ranges was $1.36 \cdot 10^{-4}$ and $2.58 \cdot 10^{-4}$ M (Table IV-3), which corresponds to 3.7 and 7 moles of copper bound per mole of ligand.

Interestingly, the shape of the Scatchard plot for polyaspartic acid is curvilinear, even though copper is complexed most likely to a single type of binding site (COO^-). The phenomenon observed here may be due to an electrostatic effect, such that the tendency of a given metal ion to interact with a particular ligand group of a macromolecule varies with the electrostatic free energy needed to change the electric charge of the macromolecule. This free energy is related to all the ionic equilibria in which the functional groups on the macromolecule partake (Stumm and Morgan 1981). In the case of polyaspartic acid, the shape of the Scatchard plot suggests that the complexation of one copper ion to the polymer decreased the tendency of a neighboring functional group to complex the next copper ion, which in turn decreased the affinity for the next copper ion.

The polyelectrolyte phenomenon has been modelled by an equation that defines an intrinsic stability constant (β_{int}) for a hypothetically uncharged polymer based on the empirically determined values of β' :

$$\beta' = \beta_{\text{int}} \exp(-2wz\bar{Z})$$

where w is an electrostatic proportionality factor, z is the charge on the metal ion, and \bar{Z} is the average molecular charge of the macromolecule (Stumm and Morgan 1981). However, the values of \bar{Z} for the conditions of analysis are needed to calculate β_{int} , and these values were not established for polyaspartic acid. Nevertheless it is reasonable that the curvilinear shape of the Scatchard plot was caused by the electrostatic effect described above and not caused by different classes of binding groups. Perhaps the same effect caused the curvilinear shape of Scatchard plots for the copper titration of the swamp samples.

3. Fluorescence Quenching

The extent of fluorescence quenching due to the addition of copper to the Waldo and Basin swamps samples is illustrated in Figure IV-15. Prior to adding copper, the relative fluorescence intensity of the Basin Swamp and the Waldo Swamp samples were 70 and 100, respectively, for measurements at identical instrument settings. For both titrations, the fluorescence values were obtained by adjusting the gain on the fluorometer such that the original sample (no added Cu) read 100 relative fluorescence units. Therefore, the fluorescence values of samples containing incremental additions of copper were a percentage of the initial fluorescence intensity.

The fluorescence of both swamp samples was quenched to a similar extent for copper additions up to $3 \cdot 10^{-5}$ M (30% quenched) (Figure IV-15). Thereafter, the Waldo Swamp water was quenched to a greater degree than the Basin Swamp sample. The maximum level of quenching

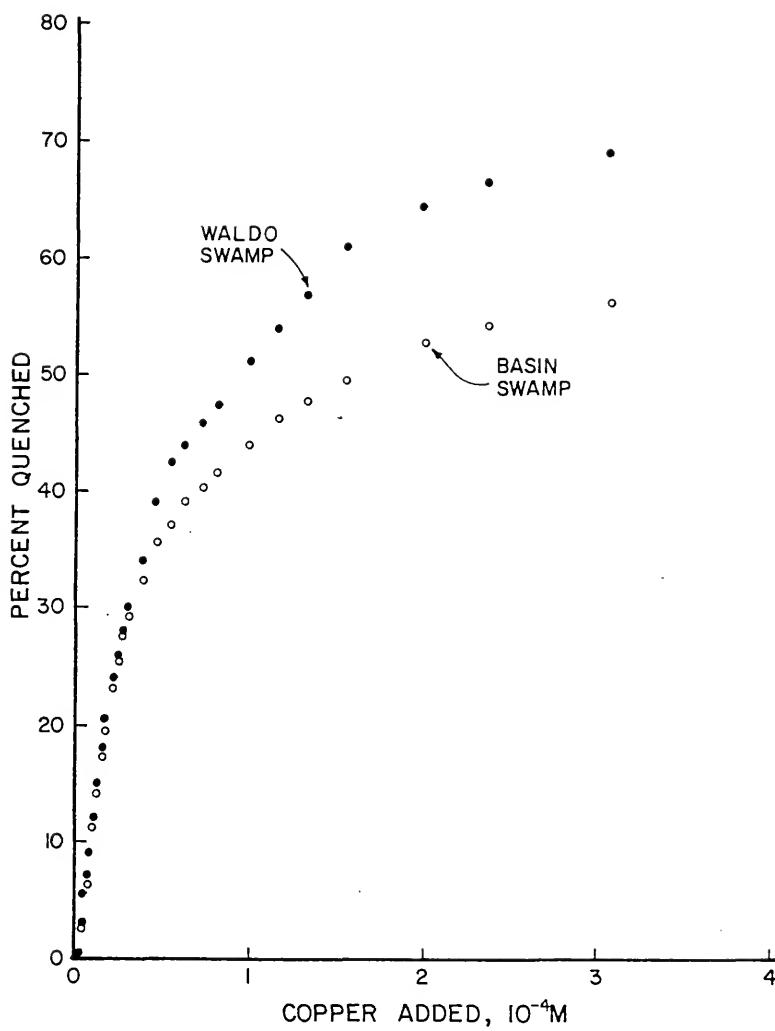


Figure IV-15. Copper titrations of swamp water samples using fluorescence spectroscopy.

achieved for each sample was 69% (Waldo) and 56% (Basin) at a copper concentration of $3.1 \cdot 10^{-4}$ M. Addition of copper to either sample at levels higher than $3.1 \cdot 10^{-4}$ M produced a floc that settled rapidly, thus preventing the measurement of complete quenching. The lower extent of quenching for the Basin Swamp sample is contrary to expectations considering the organic content of each sample (10^{-4} M, Waldo Swamp; $5 \cdot 10^{-5}$ M, Basin Swamp). For similar strength of copper binding, the lower concentration of organic matter would be more completely complexed with copper (and hence, more completely quenched) than would the higher concentration of organic matter at equivalent concentrations of copper. In addition, quenching did not approach 100% in either sample, thus suggesting that not all fluorophores in the sample were involved in copper complexation.

To convert the values of percent quenched to concentrations of copper bound, Cu_b , Saar and Weber (1980) used the relationship:

$$\% \text{ Quenched}/57 = \bar{V} = (Cu_b)/(L_t) \quad (4.5)$$

in which (L_t) is the total ligand concentration and 57 is an empirically determined coefficient. When eqn. 4.5 was applied to the data measured here, the calculated values of Cu_b were found to be greater than the analytical copper concentration; a physical impossibility. These results could be caused by a difference between samples in the proportion of fluorescence quenching by complexed copper or by a difference in actual (or assigned) molecular weights of organic matter. The latter possibility was examined by multiplying the empirical coefficient (57) by the ratio of molecular weight used here (1000), to the molecular weight (644) used by Saar and Weber (1980). The coeffic-

ient becomes 88, which still produced physically meaningless values of Cu_b for both samples. However, the value 88 determined in the manner described would be valid only if the actual molecular weights of the organics titrated by Saar and Weber (1980) and those titrated here were identical. Apparently the molecular weights or the nature of fluorescence quenching by complexed copper varies among sources.

Consequently, I evaluated the empirical constant at two arbitrary levels of total copper— $3 \cdot 10^{-5}$ and 10^{-4} M—by inserting values of Cu_b that were determined using ISE into eqn. 4.5. In the case of the Waldo Swamp water, the coefficient was 104, whereas for the Basin Swamp sample, the value was 150. Therefore, Scatchard analysis of the Waldo Swamp water titration was performed using the equation:

$$\% \text{ Quenched}/104 = \bar{V} = (Cu_b)/(L_t), \quad (4.6)$$

and the equation used for the Basin Swamp sample was

$$\% \text{ Quenched}/150 = \bar{V} = (Cu_b)/(L_t). \quad (4.7)$$

As a result of the required calibration of the fluorescence quenching data by using Cu_b values determined with ISE, the fluorescence quenching technique is not a completely independent method to determined C_L and β' .

The copper binding capacity, C_L , was determined by plotting the negative log of bound copper concentrations, pCu_b , versus the negative log of the analytical copper concentrations, pCu_t (Figure IV-16). The asymptotic values, C_L , were $7.4 \cdot 10^{-5}$ M and $2.1 \cdot 10^{-5}$ M for the Waldo and Basin swamps' samples, respectively.

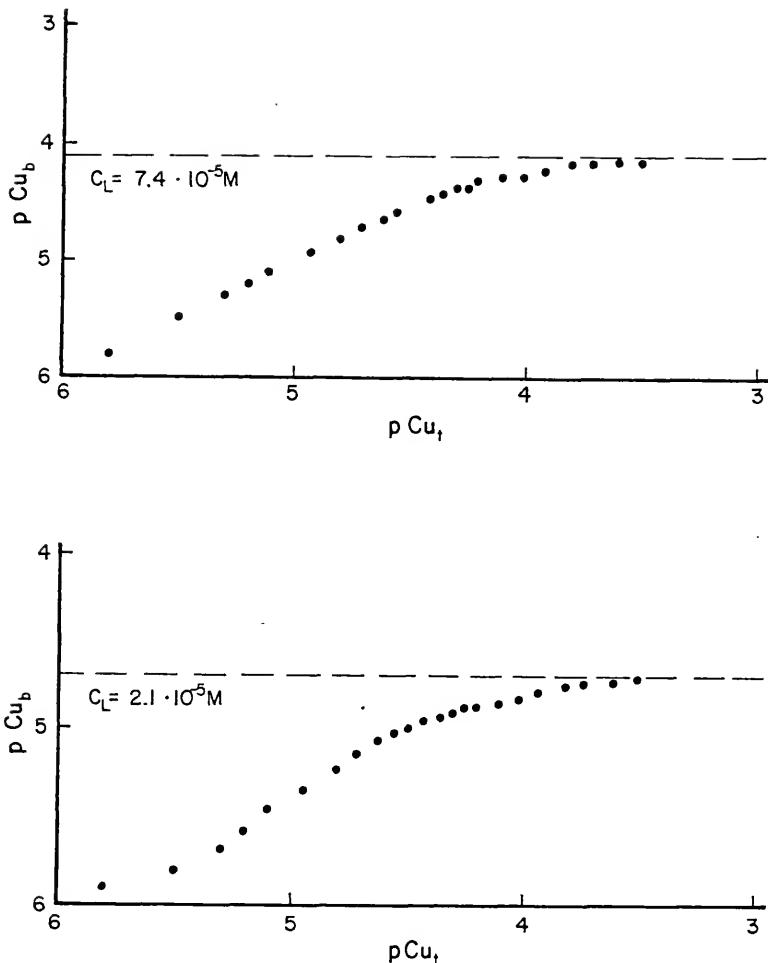


Figure IV-16. Binding curves for copper titrations of Waldo Swamp (upper) and Basin Swamp (lower) water samples using fluorescence spectroscopy.

Conditional stability constants were determined by Scatchard plots (Figure IV-17), which were segmented for comparative purposes. The lowest ranges previously selected were below the detection limits of this method. For the initial additions of copper, the relative fluorescence remained large, resulting in small differences between two large numbers. This phenomenon accounts for the high detection limit and large amount of scatter for the low values of \bar{V} in Figure IV-17.

The Scatchard plot for the Waldo Swamp water produced a β' value for the intermediate \bar{V} range (0.021–0.2) of $7.3 \cdot 10^6$ and for the \bar{V} range 0.21–0.8, β' was $1.5 \cdot 10^6$ (Table IV-4). The molarities of the two ranges were $2 \cdot 10^{-5}$ and $5.3 \cdot 10^{-5}$ M, respectively. Thus the weaker binding site complexed more than twice as much copper as the stronger site did. For the Basin Swamp sample, the stability of complexed copper was $4.8 \cdot 10^5$ for \bar{V} range 0.026–0.125 and $7.45 \cdot 10^4$ for \bar{V} range 0.126–0.4. Again, the weaker site complexed more copper ($1.15 \cdot 10^{-5}$ M) than did the stronger one ($5 \cdot 10^{-6}$ M). The magnitudes of the β' values (Table IV-4) for the two water samples range from intermediate ($\beta' = 7.45 \cdot 10^4$) to moderately strong ($\beta' = 7.3 \cdot 10^6$). As expected, the stronger complexes formed during the initial stages of the titration, and weaker binding followed. A comparison of the above β' values with those obtained from the other procedures is presented in a subsequent discussion. Cadmium and zinc were not examined by the fluorescence quenching procedure because they are not paramagnetic and hence do not quench fluorescence.

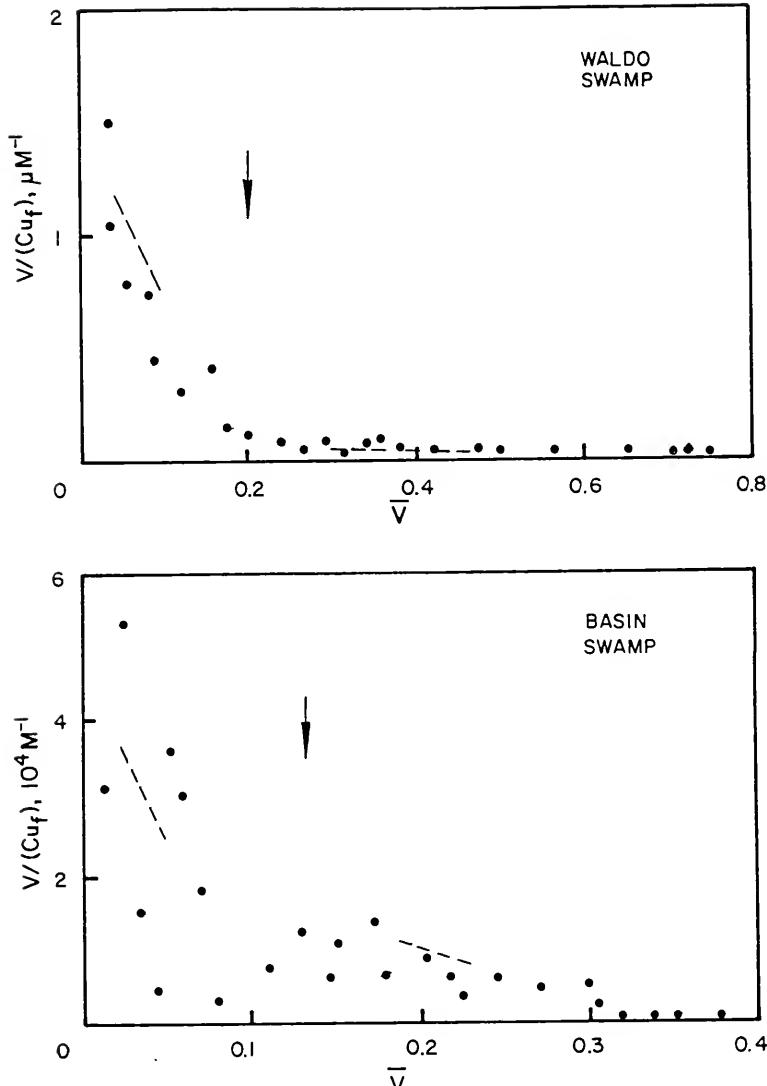


Figure IV-17. Scatchard plots for copper titrations of water samples using fluorescence spectroscopy. Arrows delimit linear segments (dashed lines).

Table IV-4. Copper binding capacities and conditional stability constants for surface water samples using fluorescence quenching.

	Copper Binding Capacity	\bar{V} Range	Molarity of V Range	β'	$\log \beta'$
Waldo Swamp	$7.4 \cdot 10^{-5}$ M	0-0.02	---	---	---
		0.021-0.2	$2 \cdot 10^{-5}$	$7.3 \cdot 10^6$	6.80
		0.21-0.8	$5.3 \cdot 10^{-5}$	$1.5 \cdot 10^6$	5.17
Basin Swamp	$2.1 \cdot 10^{-5}$ M	0-0.025	---	---	---
		0.026-0.125	$5 \cdot 10^{-6}$	$4.8 \cdot 10^5$	5.70
		0.126-0.4	$1.15 \cdot 10^{-5}$	$7.45 \cdot 10^4$	4.87

4. Continuous Ultrafiltration

Because the method of continuous ultrafiltration was developed here for metal binding studies, several preliminary experiments were performed. Prior to analyzing samples by the continuous ultrafiltration method, a solution containing only 0.1 M KNO₃ at pH 6.25 was titrated with metal to ensure that the system was functioning properly. For continuous filtration with no binding component in the cell, the following equation relates effluent concentrations of metal (M_e) to influent metal concentrations (M₀):

$$(M_e) = (M_0)[1 - (1 - \alpha)^n] \quad (4.8)$$

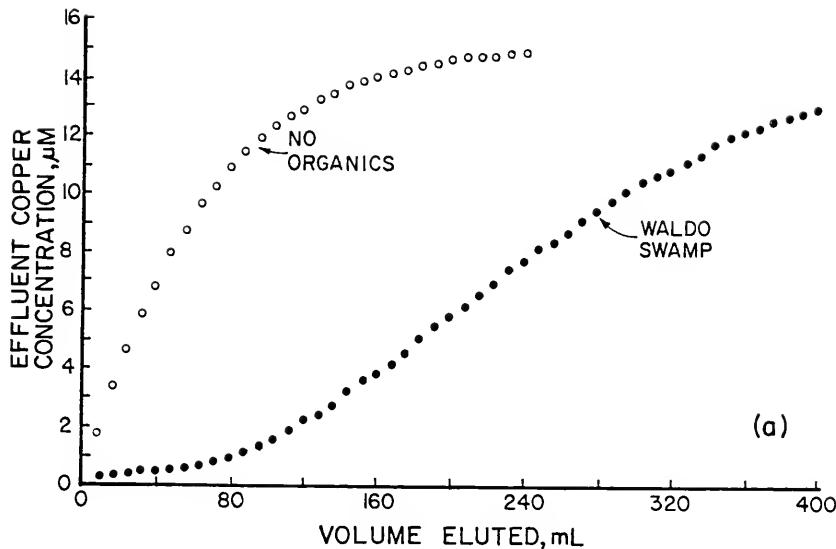
where n is the number of fractions, and α is the ratio of fraction volume to cell volume (Ryan and Hanna 1971). For the three metals examined using this technique, the theoretical values predicted by eqn. 4.8 were compared to the experimental results for cell containing no organic component. Cadmium, copper, and zinc all produced curves that were nearly identical to predicted ones (Figure IV-18), which indicates that metal rejection or binding by the membranes was not occurring.

The results plotted according to eqn. 4.8 are curvilinear, and deviations from ideal behavior are not easily detected graphically. Equation 4.8 can be rearranged to:

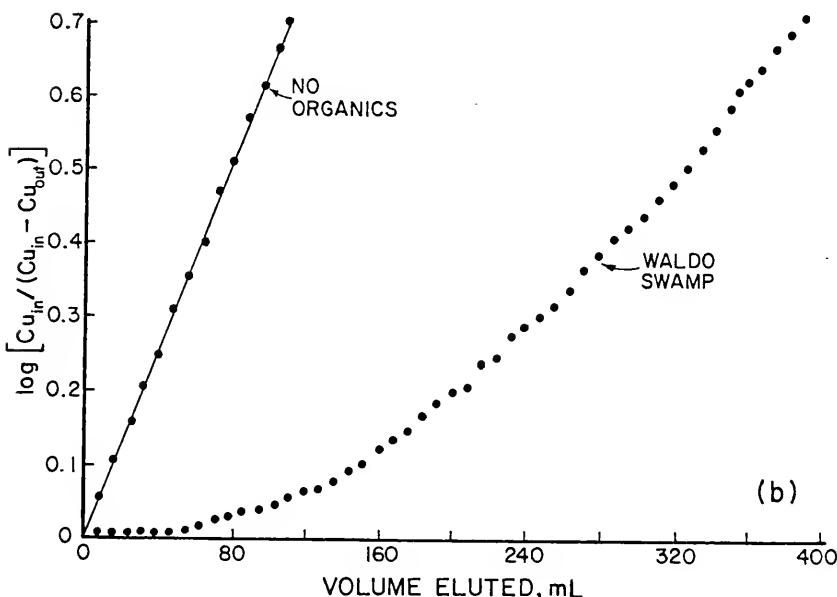
$$(M_0)/[(M_0) - (M_e)] = (1 - \alpha)^{-n}. \quad (4.9)$$

By taking logarithms of both sides, eqn. 4.9 becomes

$$\log[(M_0)/[(M_0) - (M_e)]] = -n \log (1 - \alpha). \quad (4.10)$$



(a)



(b)

Figure IV-18. Titrations of Waldo Swamp and organic-free solutions with copper using ultrafiltration. Solid line represents theoretical dilution.

Hence a plot of the left side of eqn. 4.10 versus the volume of eluant added produces a straight line for the simple dilution experiment. Figure IV-18b illustrates the theoretical line and experimental points for the same copper dilution experiment shown in Figure IV-18a. The other metals, Cd and Zn, also exhibited ideal behavior when plotted according to eqn. 4.10.

In order to perform a continuous ultrafiltration titration, the organic matter of interest must be retained by the ultrafiltration membrane. The samples and model compounds investigated were rinsed with 0.1 M KNO₃ at pH 6.25 and the cell contents were analyzed to determine the extent of retention. The results indicated that more than 95% of the organic matter in the surface water samples from Waldo Swamp and Basin Swamp were retained by an Amicon UM-10 membrane. Solutions of the polyamino acids—polyarginine, polyalanine, and polyaspartic acid—were not retained by a UM-10 membrane, and therefore a UM-02 membrane (1000 dalton cutoff) was used. The polyamino acids were retained nearly quantitatively (>90%) by the smaller pore-size filter.

The results of the copper titration of the Waldo Swamp sample (Figure IV-18) clearly show that copper was retained by the Waldo sample. However, due to the nature of the experiment, neither of these two illustrations can be used directly to quantitate metal binding ability or conditional stability constants, because for each incremental addition of metal to the cell, a fraction of the free metal is removed in the filtrate. Therefore the quantity of metal bound, M_b, was calculated for each fraction collected by using the mass-balance equation derived in section III-D-6:

$$M_b = V_{out} (M_0) - V_{cell} (M_e) - M_{out} \quad (4.11)$$

where V_{out} is the total volume of effluent collected, V_{cell} is the cell volume, (M_0) is the metal concentration in the feed solution, (M_e) is the measured metal concentration in the effluent, and M_{out} is the total amount of metal in the cell effluent. All of the titrations were evaluated using eqn. 4.11 to determine the extent of metal binding by the retained organic matter.

The binding capacity, C_L , was evaluated by plotting (Cu_b) versus elution volume for the titrations of Waldo and Basin swamps' samples (Figure IV-19). Each curve tends to an asymptotic value that can be extrapolated to obtain C_L . The Waldo Swamp water complexed 43 μmoles of copper/L, whereas the Basin Swamp sample complexed 12.3 μmoles of copper/L. The concentrations of Cu_b that are plotted in Figure IV-19 were obtained by mass balance using eqn. 4.11. The complexing capacities thus determined compare well with the concentrations of copper measured in the cell solution after the titration was complete. The directly measured levels of complexed copper were 41.4 μM and 11.7 μM for the Waldo and Basin swamps samples, respectively. Titrations using cadmium and zinc produced about equal binding capacities that were less than those for copper (Table IV-5).

The conditional stability constants, β' , of the metal-ligand complexes were evaluated by the method of Scatchard (section III-D-6), and the plots for copper with Waldo and Basin swamps' samples are illustrated in Figure IV-20. As before, the Scatchard plots were divided into three segments so that a comparison of β' values could be made among the five procedures. However, precise measurements of copper

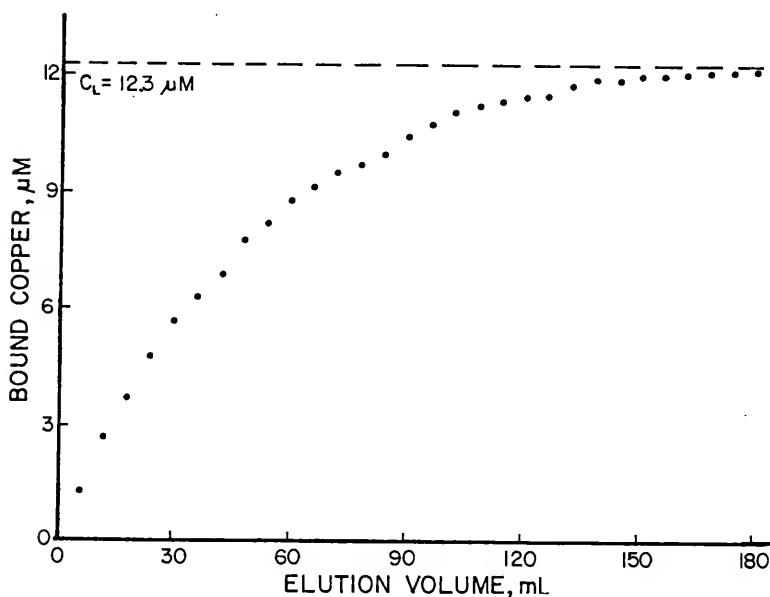
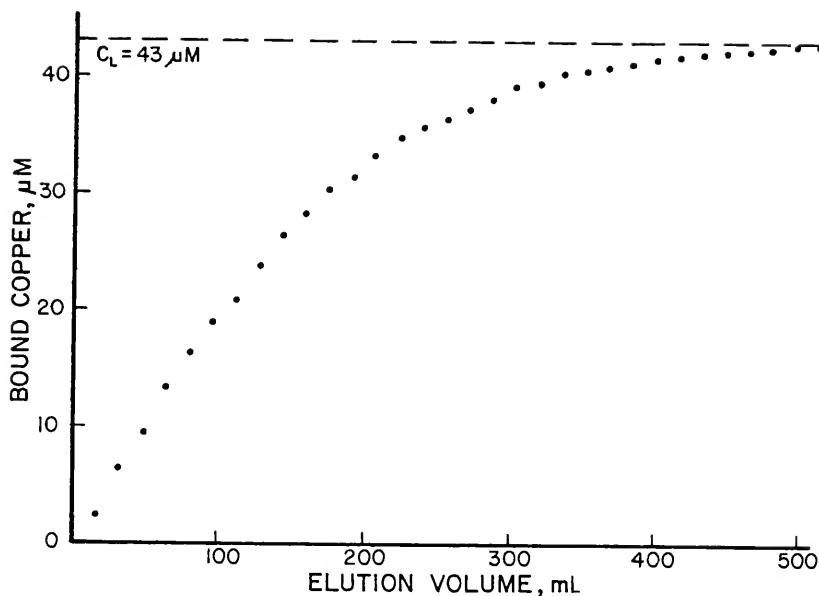


Figure IV-19. Binding curves for copper titrations of Waldo Swamp (upper) and Basin Swamp (lower) samples using ultrafiltration method.

Table IV-5. Binding capacities and conditional stability constants for water samples with cadmium, copper, and zinc using the method of ultrafiltration.

Binding Capacity	\bar{V} Range	Molarity of \bar{V} Range	β'	$\log \beta'$
<u>COPPER</u>				
Waldo Swamp	$4.3 \cdot 10^{-5}$ M	0-0.02 0.021-0.2 0.21-0.36	---	---
			$2.1 \cdot 10^{-5}$ $2.3 \cdot 10^{-5}$	$3.5 \cdot 10^6$ $5.0 \cdot 10^5$
Basin Swamp	$1.23 \cdot 10^{-5}$ M	0-0.025 0.026-0.125 0.126-0.25	---	---
			$6.85 \cdot 10^{-6}$ $8.65 \cdot 10^{-6}$	$4.7 \cdot 10^6$ $3.7 \cdot 10^5$
<u>CADMIUM</u>				
Waldo Swamp	$5.25 \cdot 10^{-6}$ M	0-0.05	$6.2 \cdot 10^{-6}$	$1.6 \cdot 10^5$
Basin Swamp	$2.3 \cdot 10^{-6}$ M	0-0.045	$3.3 \cdot 10^{-6}$	$2.3 \cdot 10^5$
<u>ZINC</u>				
Waldo Swamp	$4.9 \cdot 10^{-6}$ M	0-0.05	$5.6 \cdot 10^{-6}$	$6.0 \cdot 10^4$
Basin Swamp	$1.8 \cdot 10^{-6}$ M	0-0.04	$2.4 \cdot 10^{-6}$	$1.2 \cdot 10^5$

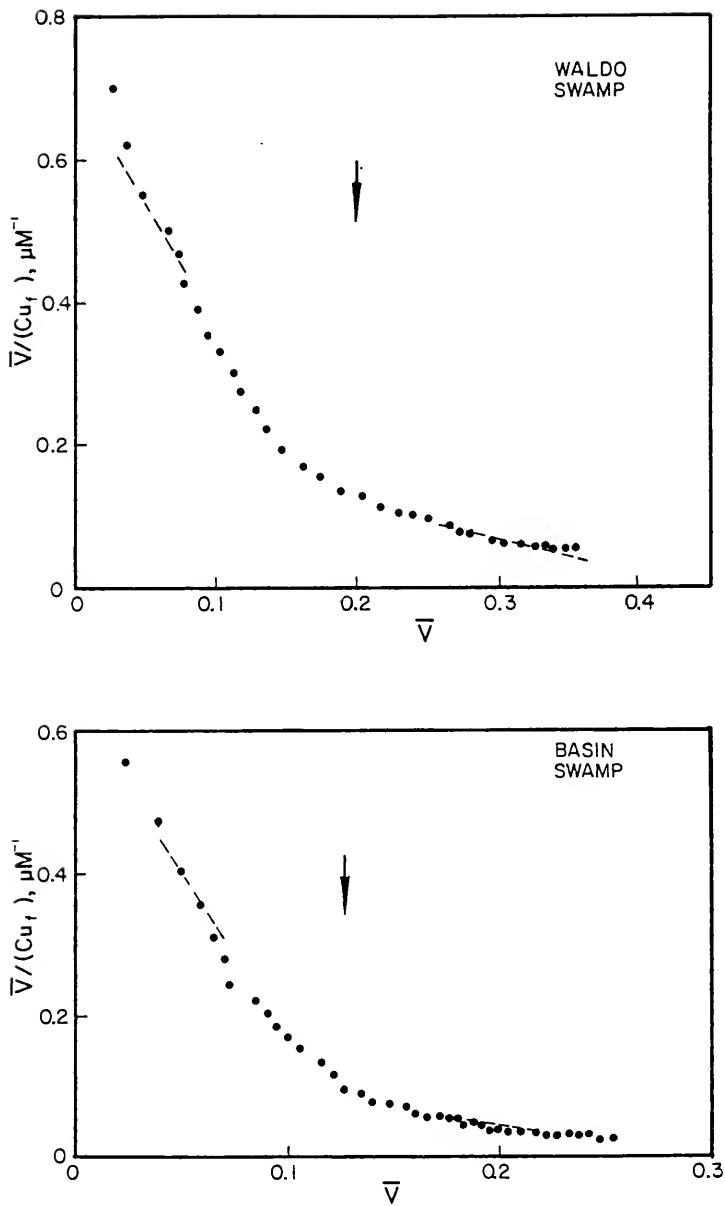


Figure IV-20. Scatchard plots of Waldo Swamp (upper) and Basin Swamp (lower) samples with copper using ultrafiltration. Arrows delimit linear segments (dashed lines).

could not be obtained below 10^{-7} M due to sorption and desorption of copper at the membrane surface. Thus, there are no values of β' for the lowest \bar{V} range. For the other two ranges, β' values were $3.5 \cdot 10^6$ and $5.0 \cdot 10^5$ for the Waldo Swamp sample and $4.7 \cdot 10^6$ and $3.7 \cdot 10^5$ for the Basin and $3.7 \cdot 10^5$ for the Basin Swamp water (Table IV-5). In both samples, the initial binding sites were complexed with copper more strongly than subsequent ones.

Scatchard plots for cadmium and zinc produced a relatively constant slope for the entire range of data for both samples, suggesting one binding site. Plots obtained for the Waldo Swamp sample with cadmium and zinc (Figure IV-21) yielded respective β' values of $1.6 \cdot 10^5$ and $6.0 \cdot 10^4$. Similar values were obtained for the Basin Swamp sample using cadmium and zinc (Table IV-5). The conditional stability constants determined for cadmium and zinc are 10–100 times lower than those obtained for copper, which is in relative agreement with other published values (e.g., Mantoura et al. 1978).

In addition to the surface water samples, three polyamino acids—polyarginine, polyalanine, and polyaspartic acid—were titrated with cadmium, copper, and zinc using the ultrafiltration method. Polyaspartic acid was the only polymer that complexed these metals.

The titration of a $3.7 \cdot 10^{-5}$ M solution of polyaspartic acid with copper resulted in a binding capacity of $3.0 \cdot 10^{-4}$ M, indicating that each mole of polyaspartic acid complexed 8 moles of copper. The Scatchard plot was divided into three sections, and, like the surface water samples, the lowest \bar{V} range for polyaspartic acid was below the limit of the procedure. The middle range ($\bar{V} = 0.26$ –4),

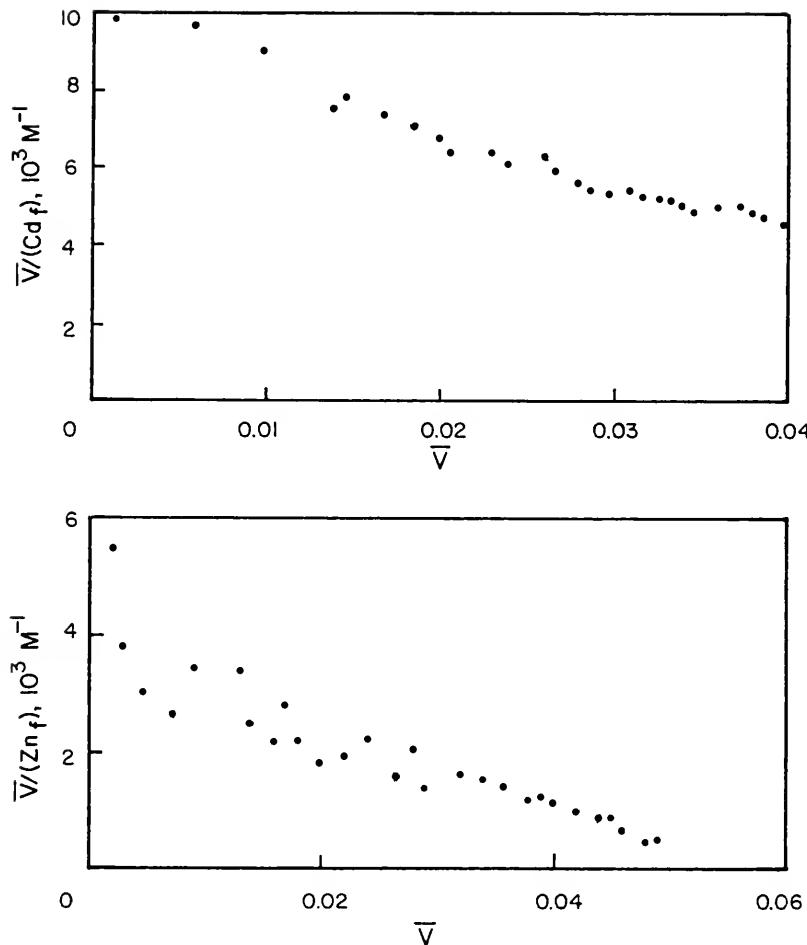


Figure IV-21. Scatchard plots for titration of Waldo Swamp water with cadmium (upper) and zinc (lower).

accounted for $1.6 \cdot 10^{-4}$ moles of complexed copper per liter with a stability of $3.2 \cdot 10^7$ (Table IV-6). The final range ($\bar{V} = 4.1\text{-}9$) had a molarity of $1.95 \cdot 10^4$ and a β' value of $2.3 \cdot 10^5$. The magnitude of the conditional stability constants indicates that copper is bound strongly at pH 6.25.

The cadmium binding capacity of polyaspartic acid was $1.2 \cdot 10^{-5}$ M, which is less than one-tenth the C_L for copper as titrant (Table IV-6). Similarly, β' was lower for the cadmium-polymer complex than for the copper complex. The Scatchard plot for the titration of polyaspartic acid with cadmium was divided into two sections and β' values were $3.1 \cdot 10^5$ and $9.0 \cdot 10^4$ for the two \bar{V} ranges (Table IV-6).

The zinc titration of polyaspartic acid indicated that the zinc binding capacity was $9.0 \cdot 10^{-6}$ M, which is similar to C_L with cadmium (Table IV-6). The stability of zinc complexation was examined by Scatchard analysis and found to be $5.0 \cdot 10^5$ and $2.2 \cdot 10^5$ for the two \bar{V} ranges (Table IV-6). These results show that cadmium and zinc complex with polyaspartic acid and naturally occurring organic matter to a similar extent and with similar stability (c.f., Table IV-5). For all samples investigated, cadmium and zinc complex with organic matter to a lesser degree than does copper.

5. Competing Ligand/Differential Spectroscopy

The method of differential spectroscopy employing salicylate as a competing ligand was developed here for determining C_L and β' for natural water samples, and therefore several assumptions about the procedure were examined to ensure its validity. To produce accurate results, salicylate must remain available (to complex copper) at the

Table IV-6. Binding capacities and conditional stability constants for homopolyamino acids with copper, cadmium, and zinc using the method of ultrafiltration.

Binding Capacity	\bar{V} Range	Molarity of V Range	β'	$\log \beta'$	
<u>COPPER</u>					
Polyaspartic Acid ($3.7 \cdot 10^{-5}$ M)	$3.0 \cdot 10^{-4}$ M 0-0.25 0.26-4 4.1-9	---	$1.60 \cdot 10^{-4}$ $1.95 \cdot 10^{-4}$	$3.2 \cdot 10^7$ $2.3 \cdot 10^5$	7.50 5.36
Polyarginine ($2.9 \cdot 10^{-4}$ M)	$< 8 \cdot 10^{-7}$ M	---	---	---	---
Polyalanine ($2.9 \cdot 10^{-4}$ M)	$< 8 \cdot 10^{-7}$ M	---	---	---	---
<u>CADMIUM</u>					
Polyaspartic Acid	$1.2 \cdot 10^{-5}$ 0-0.12 0.121-0.35	$4.2 \cdot 10^{-6}$ $9.1 \cdot 10^{-6}$	$3.1 \cdot 10^5$ $9.0 \cdot 10^4$	5.50 4.95	
Polyarginine	$< 5 \cdot 10^{-7}$ M	---	---	---	
Polyalanine	$< 5 \cdot 10^{-7}$ M	---	---	---	
<u>ZINC</u>					
Polyaspartic Acid	$9.0 \cdot 10^{-6}$ 0-0.1 0.11-0.25	$4.4 \cdot 10^{-7}$ $5.5 \cdot 10^{-7}$	$5.0 \cdot 10^5$ $2.2 \cdot 10^5$	5.70 5.34	
Polyarginine	$< 8 \cdot 10^{-7}$ M	---	---	---	
Polyalanine	$< 8 \cdot 10^{-7}$ M	---	---	---	

same effective concentration in both sample and standard. Adsorption of salicylate to organic matter present in the samples of interest would reduce the effective concentration of salicylate. Therefore the possible occurrence of adsorption was investigated by continuous ultrafiltration using a solution of salicylate (which permeated the membrane) as the eluant in a manner analogous to that described for metal binding studies (section III-D-4). The organic matter in each sample was retained by an ultrafiltration membrane (UM-10 for surface waters; UM-02 for polyamino acids), and therefore any retention of salicylate by the organics would be detected as a deficiency of salicylate in the effluent fractions. Spectrophotometric analysis of the fractions indicated that salicylate followed ideal dilution, and no binding of salicylate by the retained organic matter from each sample had occurred.

Another possible discrepancy could occur if the absorbance at 320 nm changed due to interaction of salicylate or copper with the solutes in the samples. Absorption scans of each swamp sample containing salicylate (10^{-3} M) were indistinguishable from scans for the salicylate standards. However scans of the samples (in the absence of salicylate) with and without additional copper showed a slight increase in absorbance for the Waldo Swamp sample containing additional copper, apparently due to absorption of a copper-organic complex. No absorbance for copper solutions alone occurred in the region scanned (400–280 nm). Therefore, the increase in absorbance was subtracted from the readings taken for the Waldo Swamp sample containing salicylate at corresponding levels of copper, although the correction was small (<15%). No correction was necessary for the Basin Swamp sample or for the polyamino acids.

The titration of the surface water samples using differential spectroscopy with salicylate as the competing ligand resulted in the data presented in Figure IV-22. As with the other procedures, the Waldo Swamp sample complexed more copper than did the Basin Swamp sample. The horizontal difference between the dashed line (standard curve) and each point is a graphical measure of the concentration of copper bound to naturally occurring organic matter in each sample. A plot of (Cu_b) versus the concentrations of copper added (Figure IV-23) was used to determine C_L , and values were found to be $1.3 \cdot 10^{-5}$ and $6.8 \cdot 10^{-5}$ M for Basin Swamp and Waldo Swamp water, respectively. In addition, a $3.7 \cdot 10^{-5}$ -M solution of polyaspartic acid complexed $4.2 \cdot 10^{-4}$ moles Cu/L, indicating that each mole of polyaspartic acid complexed 11 moles of copper. No binding was observed for solutions of polyarginine and polyalanine.

The concentrations of Cu_f and Cu_b were calculated using the procedure described in section III-D-5. Subsequently, a Scatchard plot was constructed (Figure IV-24), and a value of β' for each predetermined \bar{V} range was determined (Table IV-7). Both swamp samples produced similar results for similar segments of the titration. The β' values ranged from $3.0 \cdot 10^5$ to $5.2 \cdot 10^6$ for the swamp samples. Polyaspartic acid complexed copper with stability similar to that obtained for the surface water samples. The two \bar{V} ranges for polyaspartic acid have β' values equal to $2 \cdot 10^6$ and $1.5 \cdot 10^5$. For each sample titrated, the initial additions of copper were complexed the most strongly. However, the weakly bound copper represented a larger portion of the total bound copper. No data were obtained for the lowest \bar{V} range for each sample

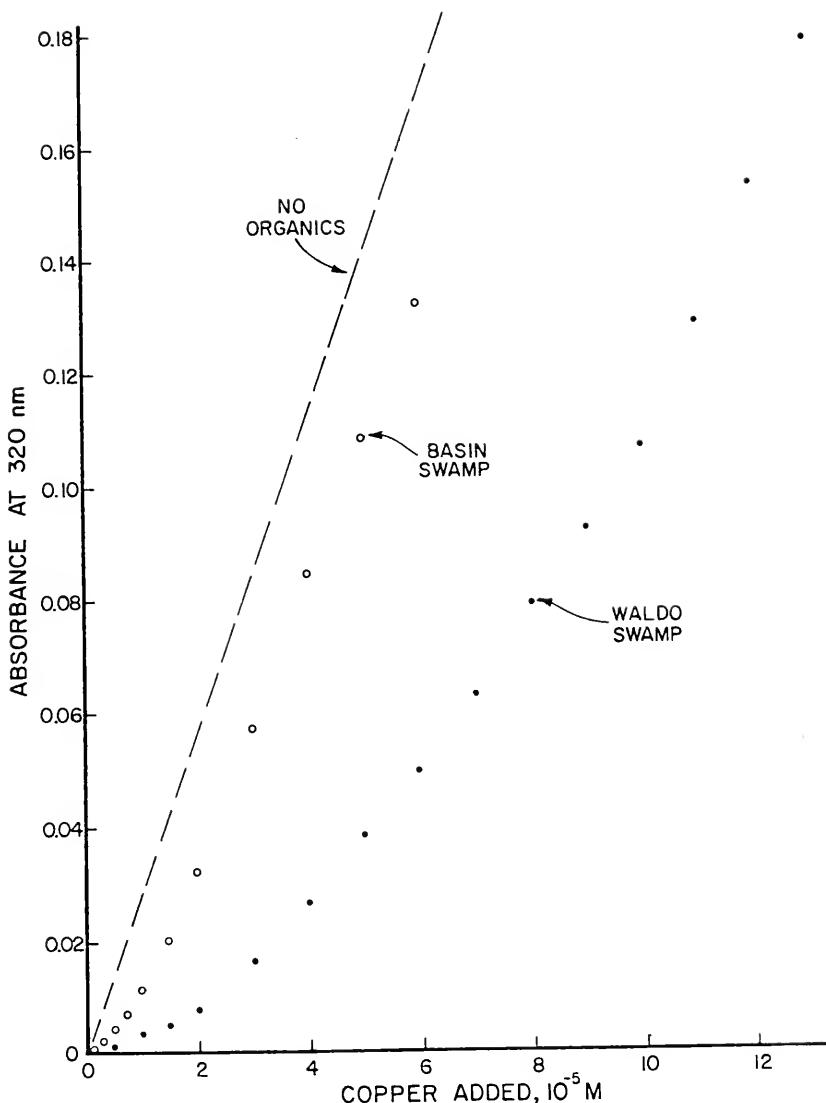


Figure IV-22. Copper titration curves of the swamp samples using competing ligand method.

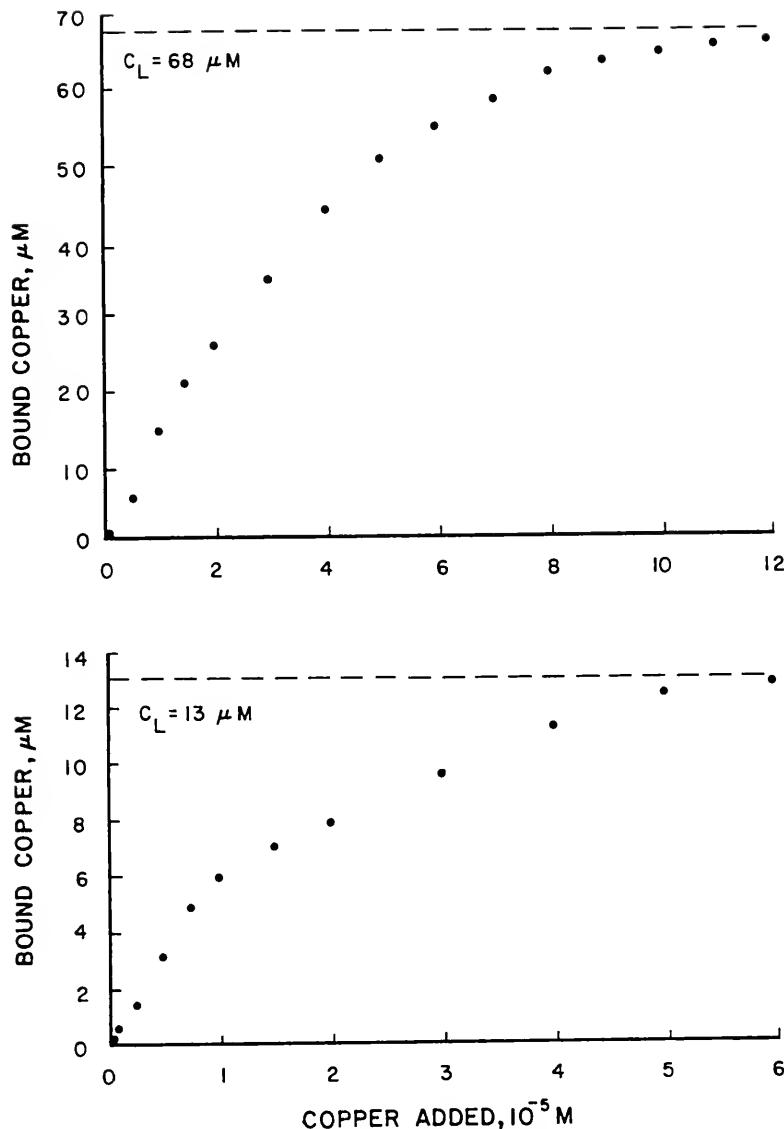


Figure IV-23. Binding curves for copper titrations of Waldo Swamp (upper) and Basin Swamp (lower) samples using competing ligand method.

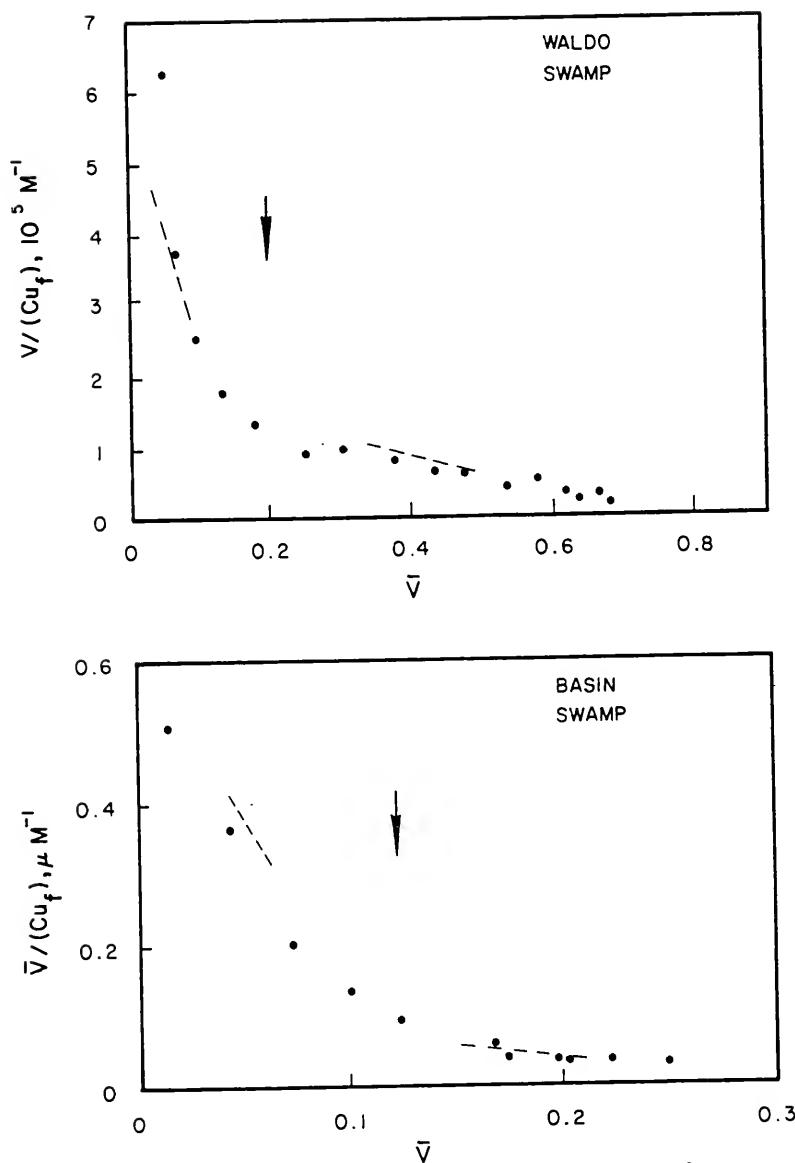


Figure IV-24. Scatchard plots for copper titrations of swamp waters using competing ligand method. Arrows delimit linear segments (dashed lines).

Table IV-7. Copper binding capacities and conditional stability constants for water samples using differential spectroscopy.

	Copper Binding Capacity	\bar{V} Range	Molarity of V Range	β'	$\log \beta'$
Waldo Swamp	$6.8 \cdot 10^{-5}$ M	0-0.02	---	---	---
		0.021-0.2	$1.5 \cdot 10^{-5}$	$4.1 \cdot 10^6$	6.61
		0.21-0.70	$5.1 \cdot 10^{-5}$	$3.0 \cdot 10^5$	5.48
Basin Swamp	$1.3 \cdot 10^{-5}$ M	0-0.025	---	---	---
		0.026-0.125	$6.2 \cdot 10^{-6}$	$5.2 \cdot 10^6$	6.72
		0.126-0.25	$8.7 \cdot 10^{-6}$	$3.5 \cdot 10^5$	5.54
Polyaspartic Acid	$4.2 \cdot 10^{-4}$ M	0-0.25	---	---	---
		0.26-4	$1.45 \cdot 10^{-4}$	$2.0 \cdot 10^7$	7.31
		4.1-10	$2.65 \cdot 10^{-4}$	$1.5 \cdot 10^5$	5.18
Polyarginine	$< 5 \cdot 10^{-7}$ M	---	---	---	---
Polyalanine	$< 5 \cdot 10^{-7}$ M	---	---	---	---

because the procedure was not sensitive to the concentrations of copper in that range.

The competing ligand method described here is restricted in some cases by its limit of detection (which is approximately 10^{-7} M $[Cu_f]$ for the conditions used). For samples producing (Cu_f) below 10^{-7} , an auxiliary competing ligand could be used to speciate copper. The auxiliary competing ligand would need a β' with copper that is larger than that for salicylate in order to compete more effectively with stronger natural organic ligands that produce such low concentrations of uncomplexed copper. The most formidable obstacle in selecting an alternate ligand is the difficulty of measuring the concentration of metal complexed to the competing ligand in the sample matrix. No acceptable alternate ligand has been found yet.

6. Comparison of Results

C_L . The copper binding capacities of the Waldo Swamp, Basin Swamp, and polyaspartic acid samples using the five techniques—ASV, ISE, fluorescence quenching, continuous ultrafiltration, and differential spectroscopy—are summarized in Table IV-8. The results of each method indicate that, for the surface water samples, the Waldo Swamp water complexed more copper than did the Basin Swamp sample. The average value of C_L was $5.45 \cdot 10^{-5}$ M for the Waldo Swamp water and $1.31 \cdot 10^{-5}$ M for the Basin Swamp sample. Of the three polyamino acids examined, only polyaspartic acid complexed copper. A solution of $3.7 \cdot 10^{-5}$ M polyaspartic acid was found to complex an average of $4.07 \cdot 10^{-4}$ moles Cu/L, which implies that 11 moles of copper bind to each mole of polyaspartic acid.

Table IV-8. Summary of copper binding capacities for surface water samples and polyaspartic acid.

Method of Analysis	Waldo Swamp	Basin Swamp	Polyaspartic Acid (3.7·10 ⁻⁵ M)
ASV	8.5·10 ⁻⁶	5.8·10 ⁻⁶	<10 ^{-7*}
ISE	7.9·10 ⁻⁵	1.4·10 ⁻⁵	5.0·10 ⁻⁴
Fluorescence Spectroscopy	7.4·10 ⁻⁵	2.1·10 ⁻⁵	---
Continuous Ultrafiltration	4.3·10 ⁻⁵	1.2·10 ⁻⁵	3.0·10 ⁻⁴
Competing Ligand/Differential Spectroscopy	6.8·10 ⁻⁵	1.3·10 ⁻⁵	4.2·10 ⁻⁴
Range	8.5·10 ⁻⁶ -7.9·10 ⁻⁵	5.8·10 ⁻⁶ -2.1·10 ⁻⁵	3.0·10 ⁻⁴ -5.0·10 ⁻⁴
Mean	5.45·10 ⁻⁵	1.31·10 ⁻⁵	4.07·10 ⁻⁴
Standard Deviation	2.9·10 ⁻⁵	5.42·10 ⁻⁶	1.0·10 ⁻⁴

*Not included in average, range, or standard deviation.

For all samples, the C_L value determined by ASV was considerably lower than the other methods. The cause of the lower values was apparently due to the reducible nature of the copper-organic complex. The validity of the ASV results remains doubtful for the reasons discussed earlier (section IV-B-1). However, excluding the ASV results, the other procedures were in close agreement.

The magnitude of C_L indicates that the swamp water samples are capable of complexing significant amounts of copper. In comparison, the copper binding capacities obtained here are greater than most of the values reported by Truitt and Weber (1981a) for seven river, lake, and swamp waters in New Hampshire. They reported C_L values ranging from 1.1 to 15 μM , with the swamp water sample complexing the most copper.

The cadmium and zinc binding capacities of each sample were examined using ASV and ultrafiltration techniques. The ASV method indicated that the cadmium and zinc complexes were reducible at the mercury electrode, and hence no complexing capacity was obtained. Continuous ultrafiltration produced C_L values for the surface waters that ranged from $1.8 \cdot 10^{-6}$ (Basin Swamp with zinc) to $5.25 \cdot 10^{-6}$ M (Waldo Swamp with cadmium). In addition, binding capacities for polyaspartic acid were $1.2 \cdot 10^{-5}$ M for cadmium and $9.0 \cdot 10^{-6}$ for zinc. In all cases, the binding capacities with cadmium and zinc were 5–10 times lower than C_L for copper. A similar trend with cadmium and copper was observed by Truitt and Weber (1981a) for surface waters in New Hampshire.

β' . A summary of the conditional stability constants obtained for copper with the organic matter in the two surface water samples and polyaspartic acid is presented in Table IV-9. Values in the lowest \bar{V}

Table IV-9. Summary of log β' values for surface waters and polyaspartic acid with copper.

\bar{V} range	ASV	ISE	Fluorescence	Analytical Technique		
				Continuous Ultra-filtration	Competing Ligand	\bar{X}
<u>Waldo Swamp</u>						
0-0.02	5.25	8.11	ND	ND	ND	7.81
0.021-0.2	5.36	6.72	6.80	6.54	6.61	6.60
0.21-0.8	ND	5.34	5.17	5.70	5.48	5.47
<u>Basin Swamp</u>						
0-0.025	6.04	7.82	ND	ND	ND	7.53
0.026-0.125	5.30	6.85	5.70	6.67	6.72	6.55
0.126-0.4	ND	5.26	4.87	5.56	5.54	5.38
<u>Polyaspartic Acid</u>						
0-0.25	ND	8.50	NA	ND	ND	8.50
0.26-4	ND	7.04	NA	7.50	7.31	7.32
4.1-15	ND	5.44	NA	5.36	5.18	5.34

ND = not detected.
NA = not analyzed.

range for each sample were obtained using ASV and ISE techniques only. The other three procedures were not sensitive enough to speciate copper for the lowest range. Values in the other two ranges agreed well among the procedures with the exception of the ASV technique. For the reasons discussed in section IV-B-1, it is apparent that the ASV procedure is not an accurate method to determine β' for copper-organic complexes (and possibly other heavy metals also). On the other hand, the results from the other four methods compared well, and, with one exception, the β' values varied within less than one order of magnitude for any given \bar{V} range of the Scatchard plot. Due to the approximate nature of β' , the results of the four methods can be considered equivalent.

The replicability of each procedure was not specifically addressed here, although precision would vary with both organic concentration and the stability of the metal-ligand complex. However, replication of one sample or another was performed for each procedure investigated here, and, in all cases, results differed by no more than 20% (and usually much less). Clearly, experimental error was not the reason for the discrepancy between results of the ASV procedure and the other four procedures. Furthermore, differences in the species of metal measured by each procedure were not responsible for the disparate results for ASV. The copper-ISE responds only to aquated Cu^{+2} , and not to the other simple inorganic complex species of copper, whereas the other four methods include inorganic species of copper in the measurement of Cu_f (i.e., copper not bound to ligand). For the conditions of analysis and the low inorganic content of each sample, the predominant form of copper not bound to ligand was Cu^{+2} in all cases. In summary, the methods of ISE, fluorescence quenching, ultrafiltration, and competing

ligand/differential spectroscopy produced values of C_L and β' that were similar among the four methods.

The magnitudes of C_L and β' for the surface water samples examined here are difficult to compare with the results of others due to the variability among samples, analytical conditions, and treatment of results. However, one study (Bresnahan et al. 1978) reported the copper titration (using ISE) of water-derived fulvic acid under conditions similar to those used here (0.1 M KNO_3 ; pH 6.0). They treated the data by Scatchard analysis, and two values of β' were reported: the initial β' was $1.3 \cdot 10^5$ (for \bar{V} range 0–0.6), and the other value was $7.0 \cdot 10^3$ (for \bar{V} range 0.6–2.0). These results are 10–100 times lower than the results obtained for Waldo Swamp and Basin Swamp water. Perhaps the differences were due to conformational changes in fulvic acid during the isolation and drying of fulvic acid performed by Bresnahan et al. (1978). Alternately the differences in values could be due to differences in the organic composition of each surface water.

In another investigation of metal binding by humic compounds in surface waters (Mantoura et al. 1978), overall stability constants for copper ranged from $10^{8.42}$ to $10^{10.21}$ for eight water samples. However, the pH of analysis was 8.0, which is considerably higher than the pH used here (6.25). A rough correlation between pH and β' values was reported recently by Buffle (1980) for a variety of humic samples. The β' values decreased approximately 10-fold for each unit decrease in pH. Therefore, by applying this relationship between pH and β' to the values reported by Mantoura et al. (1978), the range of β' values becomes $10^{6.67}$ – $10^{8.46}$ at pH 6.25. These values are similar to those obtained here for Waldo Swamp and Basin Swamp water.

CHAPTER V
COPPER BINDING BY SEWAGE, SWAMP WATER, AND
PEAT-EXTRACTABLE ORGANIC MATTER

Heavy metals entering a wetland via sewage effluent initially would be complexed (to some degree) with the organic ligands in sewage. After the sewage and swamp waters mix, redistribution of the metals among the various organic ligands is likely. Therefore, to determine which source of organic ligands complexes heavy metals to a greater extent, C_L and β' were determined for sewage and swamp water from both Waldo and Jasper. Additionally, a competitive binding experiment was performed using continuous ultrafiltration to determine the distribution of copper between the sewage and swamp waters.

A. Sewage Organics

The concentrations of soluble ($<0.5 \mu\text{m}$) organic carbon (DOC), organic nitrogen (DON), and hydrolyzable amino acid nitrogen (DHAAN) were lower for the secondary effluent (after oxidation pond) from Jasper than the septic tank overflow (septage) from Waldo (Table V-1). The percentage of dissolved organic nitrogen represented by hydrolyzable amino acid nitrogen was 36% for the Waldo septage and 16% for the Jasper sewage, indicating that a significant portion of DON is non-proteinaceous.

Table V-1. Organic content of whole and fractionated sewage from Jasper, Florida (secondary effluent), and Waldo, Florida (septage).

	Jasper Sewage	Effluent		Waldo Septage	
Entire Sample	Filtrate (UH-02)	Filtrate (UM-10)	Entire Sample	Filtrate (UM-02)	Filtrate (UM-10)
Dissolved organic carbon (mg/L)	18	18	17	28	13
Dissolved organic nitrogen (mg/L)	2.5	2.3	2.3	8.0	4.8
Dissolved hydrolyzable amino acid nitrogen (mg/L)	0.41	0.38	0.36	2.9	1.8
					1.9

Each sewage sample was ultrafiltered for subsequent metal binding studies, and the extent of membrane permeation by the soluble organics is listed in Table V-1. Nearly all of the organic matter in the treated sewage from Jasper passed through a UM-02 and a UM-10 ultrafiltration membrane. For the Waldo septage, approximately 50% of the DOC and more than 35% of the DON and DHAAN were retained by the ultrafiltration membranes. Little can be stated regarding the molecular size of these fractions because ultrafiltration membranes separate organics based on both size and charge (Amicon 1977).

Copper complexation with sewage organics was examined by determining C_L and β' using the ISE method (section III-D-2). The copper titration of each sewage sample indicated that the septage from Waldo complexed more copper ($17.5 \mu\text{M}$) than did the treated sewage from Jasper ($8.2 \mu\text{M}$) (Figure V-1). In addition, both the retained and filtered (through a UM-10 ultrafilter) fractions of Waldo septage were titrated with copper. The filtrate fraction accounted for 39% ($7.2 \mu\text{M}$) of the copper complexed by the total sample, and the retentate fraction complexed 61% ($11.5 \mu\text{M}$) (Table V-2).

Scatchard analysis of the sewage samples was performed using C_L as the molar concentration of organic matter. Each plot was segmented into two sections, resulting in two β' values for each titration. The stability of the copper-organic complexes was relatively similar among the various samples, with values ranging from $2.70 \cdot 10^4$ to $2.41 \cdot 10^5$ (Table V-2). These values of β' for the sewage organics are up to 100 times lower than the values of β' for the swamp-water organics with copper.

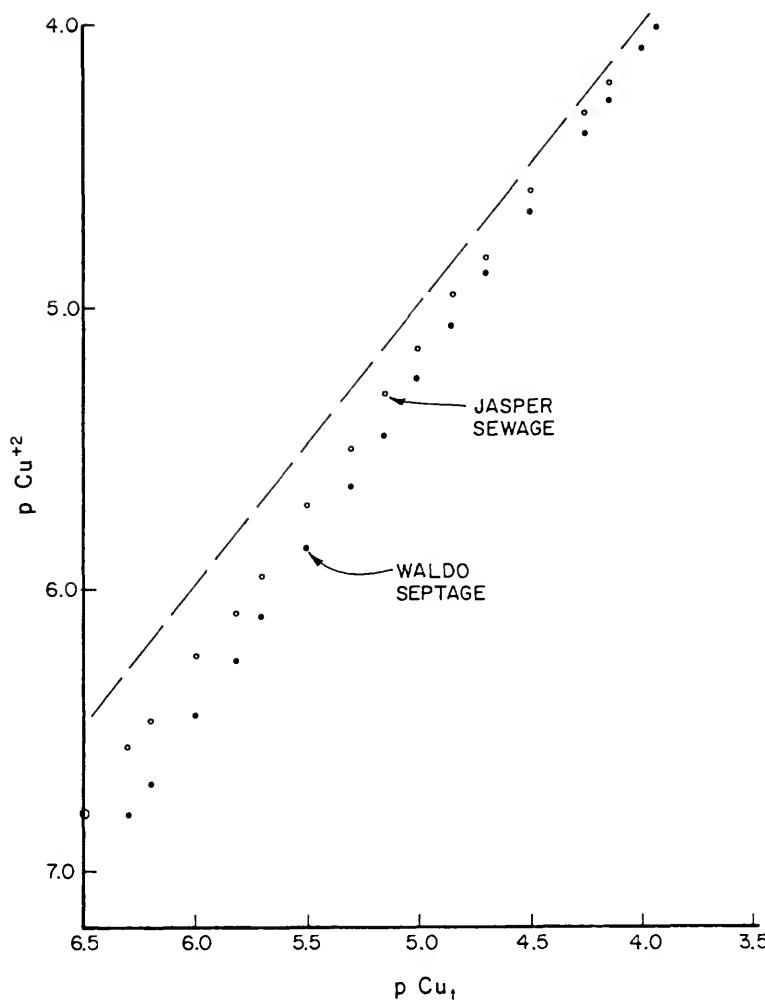


Figure V-1. Copper titration curves of Waldo septage, Jasper sewage effluent, and background electrolytes (dashed line) using ISE method.

Table V-2. Copper binding capacities and β' values for whole and fractionated sewage samples.

	C_L	\bar{V} Range	β'	$\log \beta'$
Jasper Sewage Effluent	$8.1 \cdot 10^{-6}$ M	0-0.45 0.46-1.0	$1.66 \cdot 10^5$ $2.70 \cdot 10^4$	5.22 4.43
Waldo Septage				
Entire sample	$17.5 \cdot 10^{-6}$ M	0-0.5 0.51-1.0	$2.41 \cdot 10^5$ $4.00 \cdot 10^4$	5.38 4.60
Filtrate (UM-02)	$7.2 \cdot 10^{-6}$	0-0.5 0.51-1.0	$1.51 \cdot 10^5$ $3.10 \cdot 10^4$	5.18 4.50
Retentate (UM-10)	$11.5 \cdot 10^{-6}$	0-0.5 0.51-1.0	$2.30 \cdot 10^5$ $3.85 \cdot 10^4$	5.36 4.58

B. Swamp-Water Organics

Analysis of the surface waters from Waldo Swamp and Basin Swamp for dissolved organic carbon and nitrogen showed that the surface waters were considerably higher in DOC and lower in DON than the corresponding sewage samples. The Basin Swamp water contained: DOC, 25 mg/L; DON, 0.55 mg/L; and DHAAN, 0.08 mg/L. Similarly, Waldo Swamp water had: DOC, 50 mg/L; DON, 0.75 mg/L; and DHAAN, 0.11 mg/L. The high ratios of C:N for the swamp waters are characteristic of humic compounds, which usually contain 50% carbon and 0.5–5% nitrogen (Hartenstein 1981).

The binding capacities and conditional stability constants for the surface waters were determined by five separate methods in the comparative study reported in section IV-2. The mean values of C_L for Waldo Swamp and Basin Swamp were $5.5 \cdot 10^{-5}$ M and $1.3 \cdot 10^{-5}$, respectively, and the β' values ranged from $1.5 \cdot 10^5$ to $1.3 \cdot 10^8$ and $7.4 \cdot 10^4$ to $6.6 \cdot 10^7$ for the respective samples.

In addition to the comparative study, a competitive binding experiment was performed using swamp water and sewage samples. The organics from the surface water samples were retained by a UM-10 ultrafiltration membrane, whereas most of the sewage organics passed through a UM-10 membrane. Therefore, a continuous ultrafiltration was performed in a manner similar to that described in section III-D-4. The swamp organics were placed in the ultracell and ultrafilterable sewage (i.e., filtrate from previously ultrafiltered sewage) was the eluant. Copper was added to the eluant, and pH and ionic strength were adjusted to levels used previously (pH 6.25; 0.1 M KNO_3).

The results for Waldo Swamp water with Waldo septage, and Basin Swamp water with Jasper sewage effluent were indistinguishable from similar titrations that did not include sewage organics in the eluant (see section IV-B-4). These results, as well as the values of β' obtained for each independent copper titration, indicate that the swamp-water organics complexed copper to a greater extent than did the sewage organics. The β' values for the surface waters were higher for almost all levels of total copper than the β' values for the corresponding sewage samples (c.f., Tables IV-9 and V-2). Therefore, experimental evidence shows that, for the two swamps receiving sewage, the soluble form of copper entering the swamp would be controlled by the organics originally in the swamp water.

As a result of the multiple values of β' obtained for a single titration of the swamp-water samples (see Scatchard plots in Chapter IV), the question arose as to whether several different types of ligands were responsible for the various β' values or whether metal was bound to various degrees on a single type of ligand. To determine if several distinct types of organics existed in the swamp water samples, a fractionation based on electrostatic properties of the ligand was performed. An anion exchange column of DEAE cellulose was used to adsorb organics from each of the Waldo Swamp and Basin Swamp samples. Adsorbed organics were desorbed by elution with increasing gradients of salt and pH. If a variety of organic ligands existed as discrete entities, then the more weakly bound organics were expected to desorb earlier than the more strongly bound organics. The mechanism of desorption from the DEAE column is by mass action of the anions added

to the eluant, thus displacing the organics from the ion-exchange sites.

One liter of each sample was applied individually to a column of DEAE cellulose, which was then eluted with an increasing gradient of NaCl in 0.1 M Prideaux buffer, followed by an increasing gradient of NaOH (details in section III-E). The results of desorption show two major fractions of organic matter in each sample (Figure V-2). Each of the major fractions was desalted using continuous ultrafiltration with deionized water as the eluant. Subsequently, each fraction was titrated with copper using the continuous ultrafiltration method (section III-D-4) until a complete binding curve was obtained. The binding capacities for each fraction (Table V-3) are proportional to the areas under the curves for each fraction (Figure V-2) such that about two-thirds of the organic matter (and binding capacity) was in the first fraction eluted, and approximately one-third of each was the latter fraction.

The β' values for each fraction were similar (Table V-3), which indicates that the organic matter was not chromatographed based on metal binding strength. Most likely, the separation observed in Figure V-2 was due to slight differences in desorbing ability by the increasing salt and pH gradients. Curtis et al. (1981) observed a similar trend in the separation of humics with a column of XAD resin. They found that elution of organic matter increased with slight increases in pH, and the eluted fractions were not chemically different.

The results obtained for this experiment show that the humics did not fractionate with respect to metal binding ability. Apparently, the differing values of β' observed for a single copper titration are due

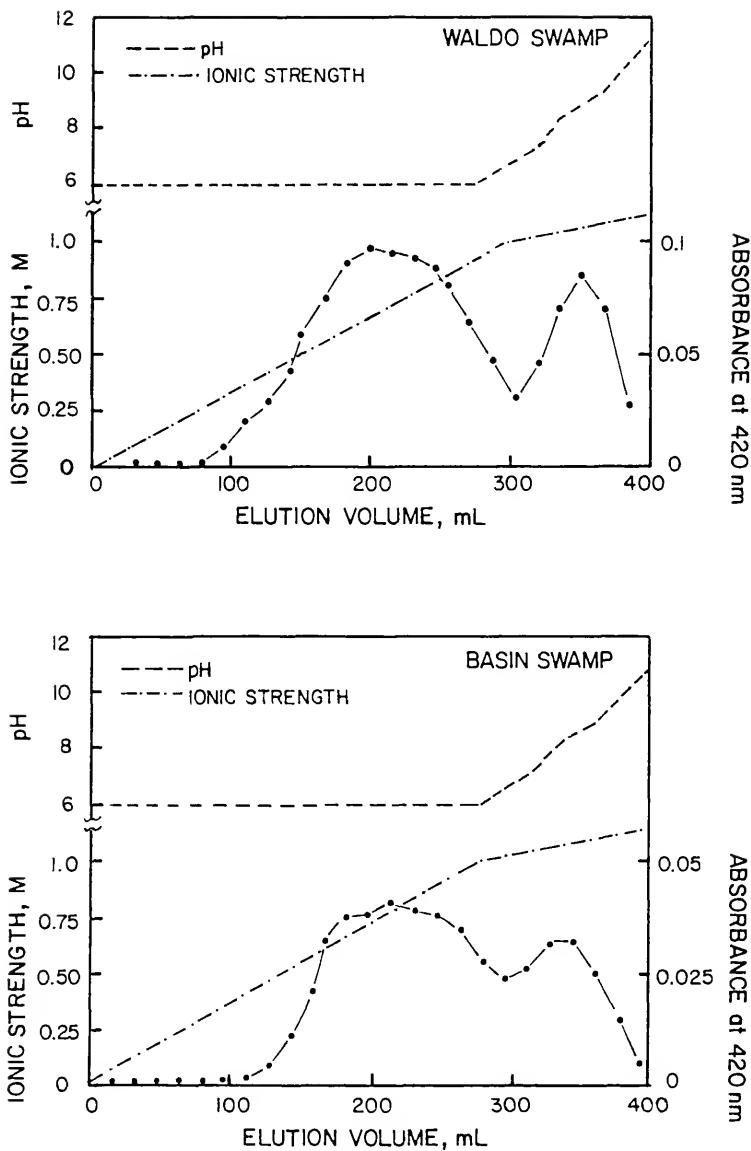


Figure V-2. Elution of adsorbed organic matter from a column of DEAE cellulose.

Table V-3. Copper binding capacities and β' values for swamp-water samples that were fractionated using DEAE cellulose.

	C_L	\bar{V} Range	β'	$\log \beta'$
Waldo Swamp				
Fraction 1	$2.9 \cdot 10^{-5}$	0-0.2 0.21-0.36	$4.5 \cdot 10^6$ $4.1 \cdot 10^5$	6.65 5.61
Fraction 2	$1.5 \cdot 10^{-5}$	0-0.2 0.21-0.36	$3.9 \cdot 10^6$ $5.7 \cdot 10^5$	6.59 5.75
Basin Swamp				
Fraction 1	$9.0 \cdot 10^{-6}$	0-0.125 0.126-0.25	$4.0 \cdot 10^6$ $3.0 \cdot 10^5$	6.60 5.48
Fraction 2	$5.1 \cdot 10^{-6}$	0-0.125 0.126-0.25	$5.2 \cdot 10^6$ $5.1 \cdot 10^5$	6.71 5.71

either to differing sites on a single ligand or to a polyelectrolyte effect (discussed in section IV-B-2).

C. Soil Organic Matter: Molecular Size Fractionation and Metal Binding Ability

The organic-rich peat sediment found in Waldo Swamp has the potential for immobilizing heavy metals by forming relatively stable metal-organic complexes. The extent and stability of heavy metal binding can be estimated by titration of a soluble sediment extract with a metal and subsequent measurement of complexed or uncomplexed metal. Originally, the intent was to fractionate the organic matter into different molecular size ranges and determine the stability of copper complexation of each size range. However, due to difficulties encountered with the molecular size separations, the copper binding capacity was determined with the original extract only. Two independent methods were employed to determine the extent and stability of heavy metal binding.

1. Molecular Size Fractionation

Molecular size fractionation was attempted using gel permeation chromatography (Sephacryl 200) using 1 M tris buffer (pH 8.6) as the eluant. The fractionation profile (Figure V-3a) of a 2 mL aliquot of the peat extract indicates that most of the organic matter was approximately 5,000 to 10,000 daltons and a small fraction was high molecular weight material (>250,000 daltons). Additionally, some organic matter eluted after the total volume of the column (V_t), and apparently this material was retarded due to hydrophobic inter-

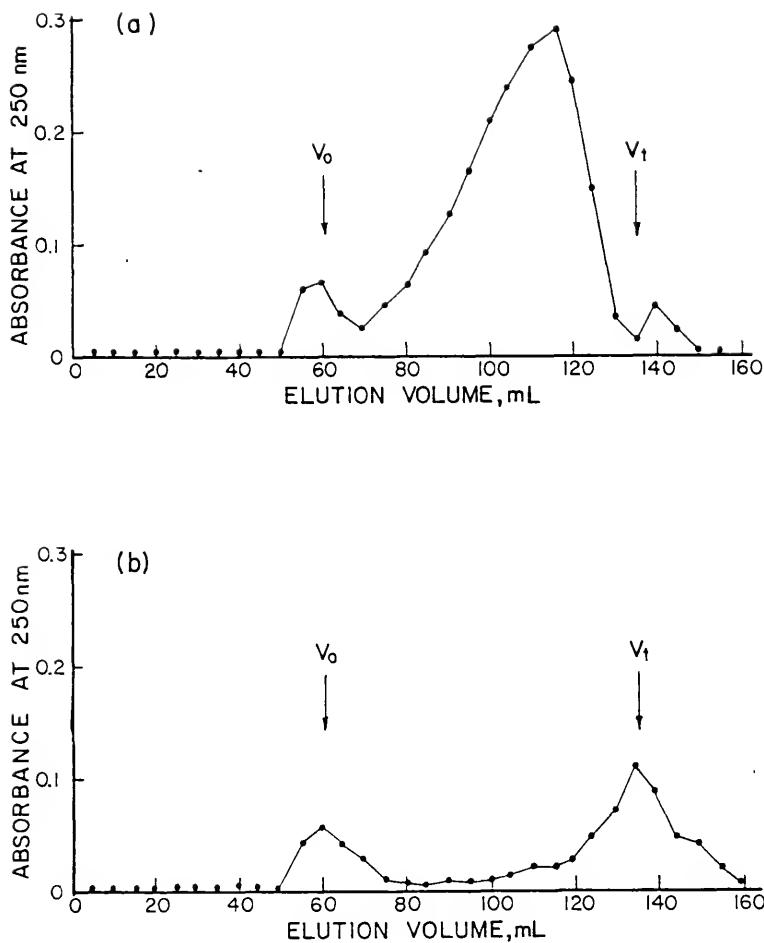


Figure V-3. Fractionation of: (a) 2 mL of peat extract, and (b) 50-60-mL fractions from (a) on Sephadex G-200 using 1 M Tris buffer at pH 8.0.

action with the gel, since some aromatic structures have an affinity for Sephadex gels (Pharmacia Corporation 1980).

The fractions containing the high molecular weight material (55–65-mL fractions) were reinjected on the column to determine if the initial chromatogram represented an accurate profile. The results (Figure V-3b) indicate that most of the high molecular weight material was excluded again, although some material eluted after the total column volume (V_t), indicating that solute-gel interactions were occurring.

Another molecular size separation procedure, ultrafiltration, was performed with the peat extract. Membranes with nominal (retention) pore sizes of 50,000 and 300,000 daltons retained more than 90% of the organic matter, suggesting that the molecular size of the organic matter was greater than 300,000 daltons (Table V-4). Due to the unlikelihood that most of the extracted organic matter was actually greater than 300,000 daltons, the experimental conditions were varied so that charge interactions with the membrane and agglomeration were minimized. Raising or lowering the pH and increasing the ionic strength produced similar results to the first experiment. Additionally the mineral content was lowered by dialyzing the extract against deionized water containing a strong cation exchange resin (Dowex 50) in order to reduce the metal concentration, especially iron and aluminum. After 48 h of dialysis with several changes of water, the mineral (ash) content was reduced from 55% to 10% (10 g ash/100 g dry wt.). The subsequent ultrafiltration experiments produced results similar to the nondialyzed sample in that more than 93%

Table V-4. Results of ultrafiltration of peat extract.

Pretreatment of Extract	Membrane Exclusion Limit (daltons)	Absorbance at 460 nm Retained	Absorbance at 280 nm Eluted	% Retained
pH = 5.0	50,000	0.40	0.01	2.10
pH = 5.0	300,000	0.39	0.01	1.95
pH = 6; 0.01 M phosphate	300,000	0.39	0.01	1.75
pH = 10.5	300,000	0.41	0.01	1.90
pH = 3.0	300,000	0.20	0.015	0.95
				Considerable loss of solute to membrane surface
pH = 6; 48-h dialysis	300,000	0.41	0.015	2.00
pH = 10.5; 48-h dialysis	300,000	0.43	0.01	1.92
				94-97
				93-96

of the extracted organic matter was retained by a 300,000-dalton membrane.

The reason for the large discrepancy in molecular weights determined by the ultrafiltration and gel filtration procedures is not readily apparent. Both procedures are known to separate compounds based somewhat on charge as well as size and shape (Swift and Posner 1971; Amicon Corporation 1977), but the use of 1 M tris buffer should minimize solute adsorption on Sephadex gels, and the large pore size ultrafilters have the least charge.

In comparison, several researchers have used colligative property techniques such as vapor phase osmometry and cryoscopy to determine molecular weights of fulvic and humic acids isolated from soil and water (Wilson and Weber 1977; Reuter and Perdue 1981; Schnitzer 1981). These workers have reported mean molecular weights in the range of 600–1000 daltons, which is 10–300 times lower than the values obtained here. Reasons for this large discrepancy in molecular weight of humic material remain in question.

2. Metal Binding of Peat Extract

Although several methods are available to quantify the interactions between organic matter and heavy metals, the two procedures selected for this experiment were the ion-exchange technique (Crosser and Allen 1976) and the ASV technique of Shuman and Woodward (1977). These methods were selected because they are simple to use, sensitive to low levels of metal, and amenable to pH values typically found in natural systems.

The ion-exchange technique consists of titrating constant levels of ligand and ion-exchange resin with various amounts of metal. The systems are equilibrated, and total soluble metal concentrations, M_{sol} (free plus complexed), are determined by atomic absorption spectroscopy. The amount of metal taken up by the resin, M_r , is calculated by the difference between the amount added initially and the amount remaining in solution at equilibrium.

Plots of M_r versus M_{sol} for titrations of samples with and without the peat extract at pH 5.9 and ionic strength of 0.01 M are illustrated in Figure V-4. For the titration of peat extract, the intercept of the two lines (S_0 and S_1') represents the available ligand concentration (C_L). The value of 7.4 μM for the extract (diluted 1:50), corresponds to a C_L of 370 μM for the original extract. Since the stock solution of peat extract contained 380 mg organic matter/L, the metal binding capacity was found to be 970 $\mu\text{moles Cu/g}$ extracted organic matter. It should be noted that C_L represents molarity of copper-titratable functional (or binding) sites and not the molarity of the ligand, i.e., more than one independent copper binding site could exist per molecule of organic matter.

The data were treated further to produce a conditional stability constant, β' , for the equilibrium between metal (M), ligand (L), and complex (ML).

In the ion-exchange experiment, the concentration of uncomplexed metal (M') was the value of M_{sol} for the titration of sample with no organic ligand. The concentration of complexed metal (ML) was determined by taking the difference between values of M_{sol}

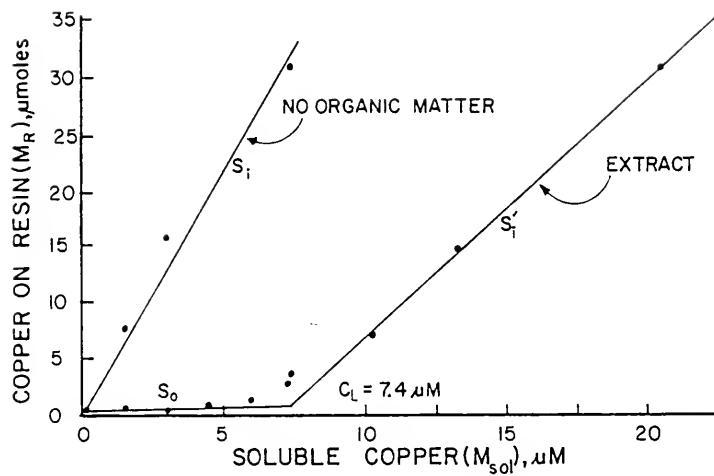


Figure V-4. Copper ion-exchange isotherms for Dowex 50X-8 and extracted organic matter.

for the two isotherms at equivalent values of M_r . By assuming that the ligand concentration is equal to the equivalence point concentration (C_L) in Figure V-4, a Scatchard plot was constructed (Figure V-5). The plot shows two nearly linear sections and thus two β' values were calculated. Initial additions of copper resulted in a $\beta' = 1.36 \cdot 10^7$ and subsequent additions of copper were bound more weakly (with $\beta' = 5.35 \cdot 10^5$).

The ASV titration procedure was used also to determine the extent of copper binding by the Waldo peat extract. A plot of copper added versus labile copper produces a titration curve from which the available ligand concentration (C_L) and the conditional stability constant (β') can be determined (section III-D-1). One of the underlying assumptions is that organically complexed copper is not measured at the mercury electrode by the ASV technique; as shown in Chapter IV (sections A and B), this assumption is not valid. However, the experiments presented here were performed prior to evaluating the ASV technique.

Results of the ASV titration of Waldo peat extract with copper indicate an available ligand concentration (C_L) of 10 μM (Figure V-6) for the diluted extract, which corresponds to 100 μM for the original extract, or 260 $\mu\text{moles Cu/g organic matter}$. The lower portion of the titration curve was replotted in the manner described previously (section III-D-1) to determine the stoichiometry of the copper-ligand complex (Figure V-7). The curve that most accurately approximates a straight line is in Figure V-7b, which indicates a 1:1 ratio of copper to ligand. By using the slope (S_L) of the line in

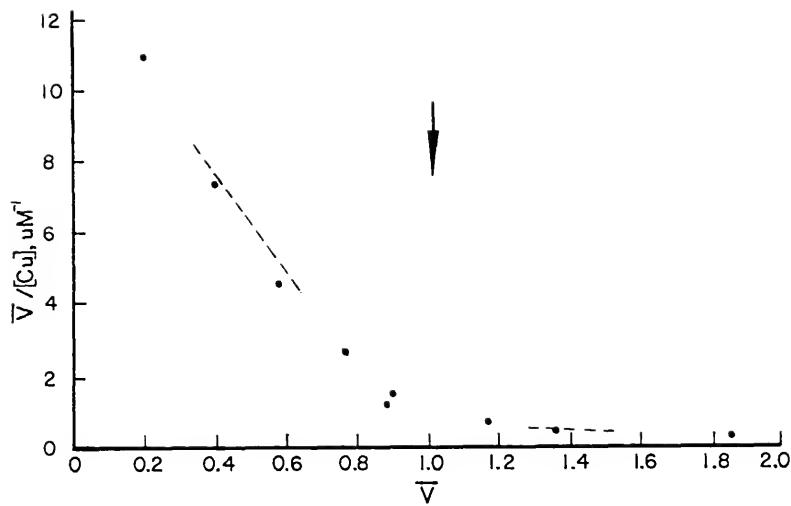


Figure V-5. Scatchard plot for the binding of copper by peat extract using ion-exchange method. Arrow delimits linear segments (dashed lines).

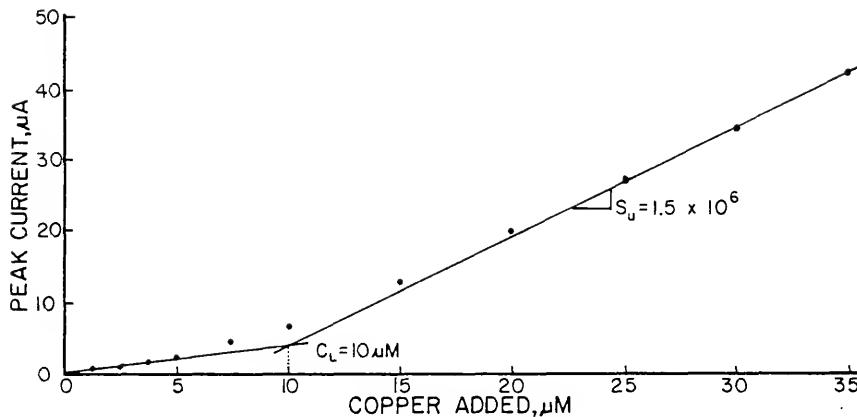


Figure V-6. ASV titration curve of Waldo peat extract at pH 5.9.

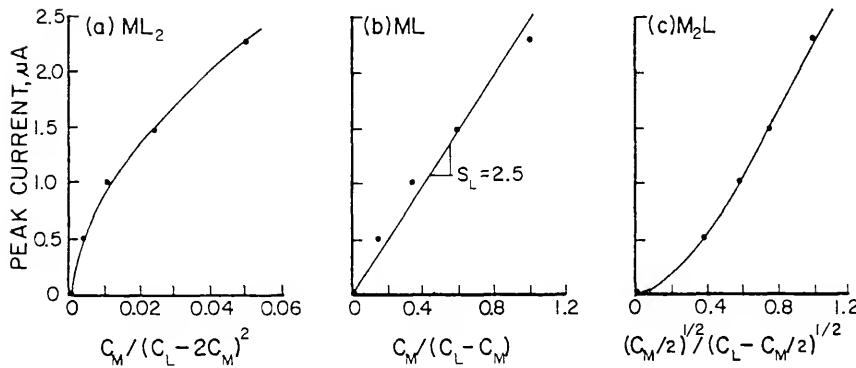


Figure V-7. Plots of copper and ligand concentrations from lower region of titration curve.

Figure V-7b ($S_L = 2.5$) and the slope of the upper region (S_u) of the titration curve in Figure V-6 ($S_u = 1.5 \times 10^6$), the conditional stability constant, β' , was calculated as follows:

$$\beta' = S_u/S_L = 10^{5.78}.$$

The magnitude of β' , which represents the mixed stability of all nonreducible complexes present in the extract, denotes moderately stable complexation of copper.

A comparison of the available ligand concentrations (C_L) determined by two independent methods reveals a higher value of C_L for the ion-exchange method (370 μM) than for the ASV technique (100 μM). Similarly, the conditional stability constants, β' , were over 10 times higher by the former method ($\beta' = 10^{7.14}$) than the latter ($\beta' = 10^{5.78}$). These discrepancies were caused, at least in part, by the ASV-lability of the copper-organic matter complex at the microzone surrounding the mercury electrode used for ASV analysis. This phenomenon has been observed using the ASV technique with model compounds in subsequent studies reported here (section IV-A). Most probably, the values of C_L and β' obtained by the ion-exchange technique approximate the true values more closely than does the ASV method.

The conditional stability constants obtained for humic substances with copper in this study and by others are summarized in Table V-5. Variations of β' values occur both with pH and ionic strength, with larger values occurring at high pH and low ionic strength. Under the conditions used in this study, the values of β' obtained for Waldo peat are similar to those reported for copper with humic

Table V-5. Conditional stability constants (β') for copper and soil-derived humic material.

Sample Origin	log β'	pH	Ionic Strength (M)	Method	Investigator
Waldo Swamp	7.14 5.73	5.9	0.01	Ion-exchange	This study
Waldo Swamp	5.78	5.9	0.01	ASV	This study
Peat	8.51* 7.16**	8.0	0.01	Gel chromatography	Mantoura and Riley (1975)
Armadale podzol	7.82	7.6	0.01	Ion-exchange	van den Berg and Kramer (1979)
Armadale podzol	5.78	3.5	0.1	Ion-exchange	Schnitzer and Skinner (1966)
Armadale podzol	8.69	5.0	0.1	Ion-exchange	Schnitzer and Skinner (1966)

*"Strong" site.

**"Weak" site.

substances of other origin. The extent of copper binding by the extracted organic matter from Waldo peat can be extrapolated to estimate the metal binding capacity of the sediment on a volumetric basis. Since C_L was found to be 260-970 $\mu\text{moles Cu/g}$ organic matter, and the peat sediment contained 88 kg organic matter/ m^3 sediment, the peat is able to bind 1.5 to 5.5 kg Cu/ m^3 of sediment due to the organic matter alone. The conditional stability constants indicate moderately stable complexation of copper by the organic matter. Clearly, the sediments are a significant sink for copper and other heavy metals of similar or greater binding strengths.

CHAPTER VI FATE OF HEAVY METALS IN A WETLAND ECOSYSTEM

In this chapter, the results of heavy-metals research for two field studies and a laboratory study using model ecosystems are discussed. An investigation of Basin Swamp near Jasper, Florida, was conducted for a 2-year period (January 1979—December 1980) to determine the efficiency and environmental effects of using cypress strands for tertiary treatment of wastewater. The study included research on nutrient cycling, public health aspects, and the fate of heavy metals. In a subsequent investigation, a portion of a swamp in Waldo, Florida, was cordoned off with fiberglass walls, and sewage spiked with heavy metals was pumped into the plots. Bureaucratic delays in getting power to the site, vandalism, and a severe drought hindered the Waldo study, but some data were collected regarding the fate of added metals. In addition to the field monitoring, a laboratory study was conducted using model ecosystems (microcosms) in which the rate of metal immobilization was investigated under various conditions.

A. Heavy Metals in Basin Swamp

Table VI-1 presents a summary of the analytical results for heavy metals in the effluent from the Jasper sewage treatment plant and the receiving waters of Basin Swamp. Raw data are presented in Brezonik et al. (1981). Levels of heavy metals entering the swamp from

Table VI-1. Summary of heavy metal data for Jasper sewage treatment plant and Basin Swamp (see Figure III-1 for map of stations).

Station	Copper*	Nickel*	Zinc*	Cadmium*	Lead*
12/04/78 2 (effluent) 7	3.0 <1.0	<2 <2	<20 <20	0.3 0.3	---
02/09/79 2 (effluent) 7	4.5 1.5	<2 <2	<20 <20	0.7 1.0	---
06/21/79 2 (effluent) 7	<1.0 <1.0	<2 <2	20 10	0.7 <0.1	1.0 2.0
09/27/79 2 (effluent) 7	3.0 3.0	<2 <2	20 35	0.1 0.1	2.0 2.0
12/17/79 2 (effluent) 7	2.0 1.0	<2 <2	25 10	0.4 0.1	<1.0 1.0
02/21/80 2 (effluent) 7	1.6 <1.0	<2 <2	30 10	<0.1 <0.1	2.0 1.0
04/15/80 2 (effluent) 7	1.0 <1.0	<2 <2	10 30	<0.1 <0.1	<1.0 1.0
07/27/80 2 (effluent) 7	<1.0 1.5	<2 <2	20 10	0.1 <0.1	<1.0 1.0
10/29/80 2 (effluent) 7	2.0 1.5	<2 <2	30 20	<0.1 <0.1	1.0 1.0

*All values in $\mu\text{g/L}$.

the sewage treatment plant were very low throughout the study period. Concentrations of lead, copper, cadmium, and nickel were near the limits of detection (0.1–2.0 µg/L) in both the effluent and swamp water. Zinc, which typically is a more abundant element, generally was found at levels below 35 µg/L in the effluent and swamp water. Similarly, the groundwater test wells were found to contain low levels of heavy metals (Table VI-2). Because of the low input concentrations of heavy metals, no spatial trends in metal concentrations were observed in the swamp. Hence, no conclusions can be stated from the routine monitoring data with regard to the effectiveness of the swamp system in removing heavy metals from wastewater.

In order to determine the temporal variability of heavy metal concentration in the sewage treatment plant effluent, two additional monitoring efforts were conducted. First, the diel variability was investigated by collecting a series of samples taken at the influent and effluent of the treatment plant during a 24-h period. The results showed that levels of heavy metals entering and leaving the sewage treatment plant were consistently low during the 24-h period (Table VI-3). In the second investigation, seven daily samples were collected from the effluent of the sewage treatment plant to determine the weekly variability of the heavy metal concentrations (Table VI-4). The results were consistently low throughout the week, thus indicating that intermittent discharge of heavy metals from the sewage treatment plant was not occurring.

The levels of heavy metals discharged from the Jasper treatment plant were 10–100 times lower than the values reported for treated domestic sewage in New York and Los Angeles (Chen et al. 1974; Klein et

Table VI-2. Concentrations ($\mu\text{g/L}$) of heavy metals in groundwater test wells (see Figure III-2 for locations of wells).

	Lead	Copper	Zinc	Cadmium	Nickel
02/21/80					
1	1.0	<1.0	<10	<0.1	<2
2	2.0	<1.0	10	<0.1	<2
3	<1.0	<1.0	10	<0.1	<2
9	1.0	<1.0	30	<0.1	<2
11	<1.0	<1.0	30	<0.1	<2
12	1.0	<1.0	<10	<0.1	<2
04/15/80					
1	<1.0	<1.0	10	0.1	<2
2	<1.0	1.0	30	<0.1	<2
3	3.0	2.0	20	0.2	<2
9	2.0	<1.0	20	<0.1	<2
11	<1.0	<1.0	40	0.1	<2

Table VI-3. Concentrations ($\mu\text{g/L}$) of heavy metals in influent and effluent of Jasper sewage treatment plant collected during a 24-h period.

	Copper	Nickel	Zinc	Cadmium	Lead	Manganese
05/23/79						
Influent (mean of 25 samples)	3.7	<2	33	0.5	2.0	27
Secondary settling basin (mean of 9 samples)	0.4	<2	13	0.2	1.8	18
Oxidation Pond effluent (mean of 9 samples)	0.5	<2	12	0.3	1.7	9

Table VI-4. Concentrations ($\mu\text{g/L}$) of heavy metals in eight daily samples from sewage treatment plant effluent (oxidation pond effluent).

Date	Lead	Copper	Zinc	Cadmium	Nickel
8/10/79	1.0	4.0	50	0.1	<2
8/11/79	2.0	2.0	30	0.4	<2
8/12/79	1.0	3.0	50	0.1	<2
8/13/79	1.0	<1.0	25	0.1	<2
8/14/79	4.0	1.0	30	0.1	<2
8/15/79	<1.0	3.0	30	0.4	<2
8/16/79	3.0	<1.0	30	<0.1	<2
8/17/79	4.0	<1.0	30	0.2	<2

al. 1974). Reasons for the very low levels of metals in Jasper sewage are not readily apparent. Domestic sources of copper and zinc, for instance, include water pipes, foods and other consumables (such as some shampoos, vitamins, and drugs), and, hence, one would expect to find these metals in domestic sewage. Moreover, Williams et al. (1974) reported atmospheric levels of heavy metals to be up to 100 times higher in urban areas than in remote ones; hence, higher levels of metals are expected in surface runoff, which often enters the sewage treatment facility. Nevertheless, we found very low levels of heavy metals in Jasper sewage, in agreement with other investigations of heavy metals in domestic sewage in Florida (Klein 1976; Boyt et al. 1977; Carriker 1977). Because of the low concentrations (near detection limits) in sewage effluent, no conclusions can be made regarding the fate of heavy metal inputs to cypress swamps. On the other hand, it is apparent that, at least in the case studied here, heavy metal pollution of the receiving (swamp) water by sewage effluent is not a problem.

B. Fate of Metals Added to Waldo Swamp

The results of the samples collected at the study site in Waldo (see Chapter III-B for site description) indicate that a large portion of the added metals were immobilized by the swamp. Concentrations of the four metals at the discharge pipe, which, for the metals corridor, was directly after addition of metals, and at the end of the 40-m plots are summarized in Table VI-5. In all cases, the levels of metals

Table VI-5. Concentrations of heavy metals in surface water from experimental site in Waldo, Florida.

Sampling Date and Station	Metal Concentration ($\mu\text{g/L}$)			
	Cadmium	Copper	Manganese	Zinc
03/03/81				
Discharge Pipe (metals side)	3.2	30	70	87
Plot Effluent (metals side)	1.3	10	42	25
Reference	0.05	5	40	10
Control Plot (mean)	0.2	10	29	33
03/10/81				
Discharge Pipe (metals side)	18	133	270	250
Plot Effluent (metals side)	1.8	17	47	43
Reference	0.5	5	27	28
Control Plot (mean)	0.5	5	28	37
03/25/81				
Discharge Pipe (metals side)	9.1	51	100	110
Plot Effluent (metals side)	1.5	10	35	35
Reference	0.5	5	30	20
Control Plot (mean)	0.3	6	16	22
03/31/81				
Discharge Pipe (metals side)	12	105	170	170
Plot Effluent (metals side)	1.7	5	50	30
Reference	0.3	1	30	15
Control Plot (mean)	0.5	2.5	22	21
04/11/81				
Discharge Pipe (metals side)	11	115	280	100
Plot Effluent (metals side)	0.8	4	50	20
Reference	0.2	7	60	20
Control Plot (mean)	0.4	9	45	37

declined significantly within the plots, indicating metal immobilization in the peat substrate.

A typical example of the rate of immobilization of metals observed in the experimental plots is illustrated in Figure VI-1 for the samples collected March 10, 1981. Concentrations of all four of the added metals—copper, cadmium, manganese, and zinc—decreased by 80–90% within the 40-m study site. The same samples contained constant levels of sodium (in this case an environmentally conservative parameter), which shows that dilution was not causing the observed decrease. The metals in the control plot (receiving septage only) were consistently low during the same sampling period, with values similar to those in the reference area. At the 40-m station, the levels of copper, manganese, and zinc in the metals-enriched plot appeared to be within acceptable limits for surface water, but cadmium, at the concentrations found (0.8–1.8 µg/L), could be harmful to sensitive aquatic life such as cladocerons and salmonid fish (U.S. EPA 1976). However, the levels of metals in several effluent samples exceeded the most recent freshwater quality criteria published by U.S. EPA (1980). The criteria, which are related to hardness, state that the concentrations must not (at any time) exceed 3.0, 22, and 320 µg/L for Cd, Cu, and Zn, respectively, at a hardness level of 100 mg CaCO₃/L. In all cases, the concentrations of Cd, Cu, and Zn at the 40-m station were below these limits. However, 24-h average criteria were established also, and on several occasions copper and cadmium were above the average limits (0.025, 5.6, and 47 µg/L for Cd, Cu, and Zn, respectively, at a hardness of 100 mg CaCO₃/L) (Table VI-5). Nevertheless, the limits established by EPA are most stringent, and the levels of metals in the water leaving the

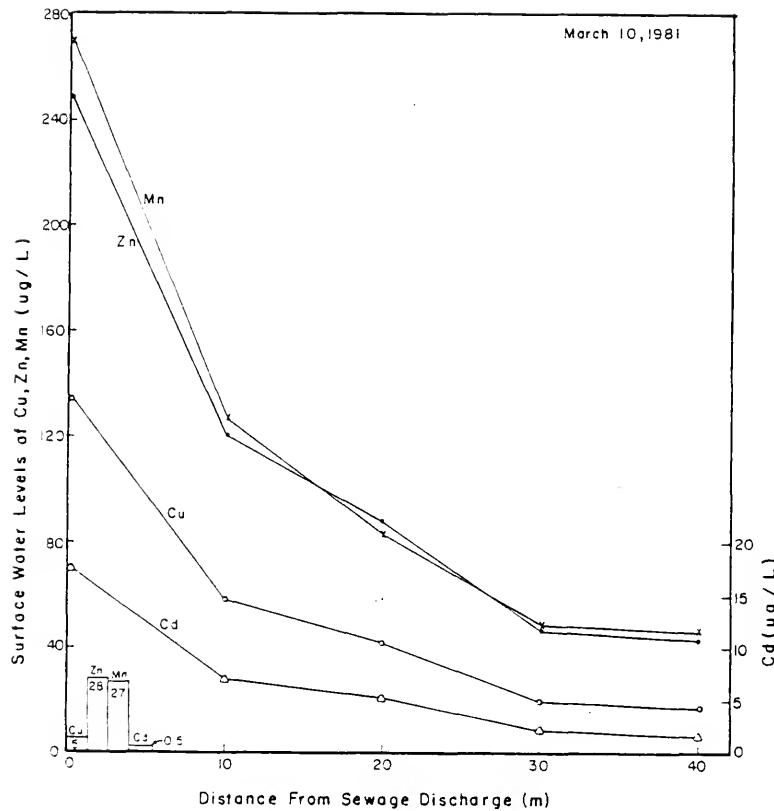


Figure VI-1. Concentrations of heavy metals in swamp receiving sewage spiked with heavy metals. Histogram represents levels at reference site. Note different scale for Cd.

site are not likely to be harmful to aquatic life, especially considering that the surface water contains a high level of organic matter and particulates, which are capable of complexing and adsorbing metals.

Within the confines of the experimental plot, concentrations of the heavy metals were reduced from the initial enriched levels to levels close to those considered acceptable for discharge to surface water. For a pumping rate of 20 L/min into a 0.04-ha plot, the hydraulic loading rate would be $720 \text{ m}^3/\text{ha}\cdot\text{day}^{-1}$ (or 7.2 cm/day). Immobilization rates of the metals were calculated (using the lowest removal) to be a minimum of 7.2, 36, 72, and 72 g metal/ $\text{ha}\cdot\text{day}^{-1}$ for Cd, Cu, Mn, and Zn, respectively. However these rates are based on a short-term study, and long-term rates are untested and unknown at this time. It is possible that after a period of time the system could become saturated with metals, resulting in reduced metal-immobilization rates.

C. Microcosm Study

Several factors control the solubility and hence availability of heavy metals. For instance, low redox conditions allow heavy metals to exist as insoluble sulfides, whereas high redox potentials favor oxidation of iron and manganese, which are known to act as metal scavengers (Jenne 1968; Khalid et al. 1977). In addition, exchangeable cations, such as calcium and magnesium, and soluble organic matter have been reported to affect the adsorption of heavy metals to exchange sites on organic and inorganic debris (Bittell and Miller 1974). Therefore, a laboratory study was performed using microcosms (i.e., small model eco-

systems) to determine the effect of certain parameters on the rate of metal immobilization. The parameters most likely to alter the state of the metals—copper, cadmium, manganese, and zinc—are DOC, calcium, iron, sulfide, and pH. The experiment consisted of varying the levels of the five parameters listed above, adding the four heavy metals, and analyzing the water for each metal. Sampling was done at 24-h intervals over 5 days (120 h). Both filtered (through 1- μm polycarbonate filters) and unfiltered samples were collected; because only slight differences were observed between filtered and unfiltered samples, only the results for unfiltered samples are reported. Additionally, systems containing only surface water were examined concurrently with the microcosms, which contained both water and peat sediments.

An example of the rate of immobilization of copper, cadmium, manganese, and zinc for the conditions: DOC, 50 mg/L; pH, 7.0; iron, 0.3 mg/L; calcium, 4.0 mg/L; and sulfide, <0.1 mg/L is shown in Figure VI-2. The other treatments produced curves similar to those in Figure VI-2 in that the aqueous concentrations of metals decreased rapidly during the first 24-h period after spiking, followed by a more gradual decline in metal concentration during the remainder of the experiment. However, the rates of immobilization varied with some treatments (Table VI-6). It should be noted that a strong similarity existed between the field monitoring data and the results from the microcosm experiment for similar treatment conditions (c.f., Figure VI-1).

The plots of metal concentration versus time (e.g., Figure VI-2) were linearized by transforming the data so that the results of the

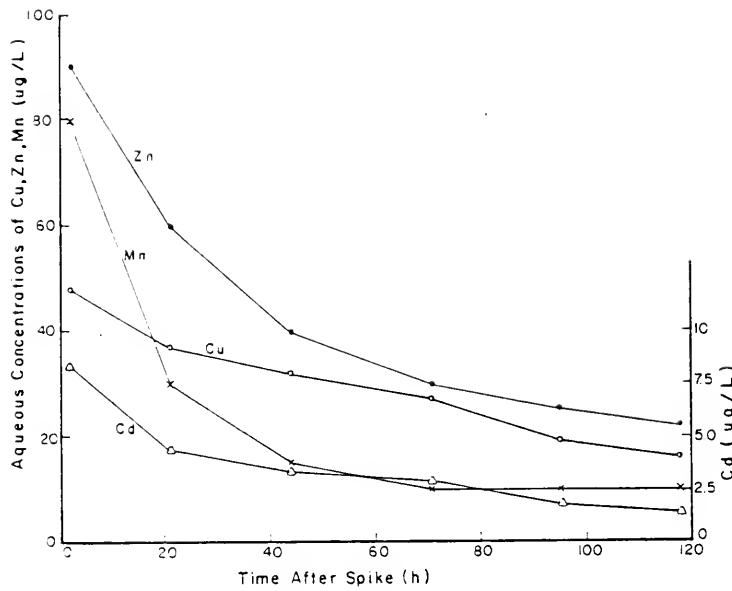


Figure VI-2. Concentrations of heavy metals in microcosm spiked with metals. Note different scale for Cd.

Table VI-6. Factors, levels, treatments, and percent reductions of heavy metals for microcosm experiment.

DOC	pH	TREATMENTS			PERCENT REDUCTION			
		Calcium	Iron	Sulfide	Cadmium	Copper	Manganese	Zinc
1	50	7.0	40	5.0	5.0	75	38	89
2	50	7.0	40	5.0	<0.1	91	60	92
3	50	7.0	40	0.3	5.0	76	34	69
4	50	7.0	4.0	5.0	5.0	72	41	80
5	50	4.0	40	5.0	5.0	40	35	24
6	50	7.0	40	0.3	<0.1	90	61	90
7	50	7.0	4.0	0.3	<0.1	93	61	82
8	50	4.0	4.0	5.0	5.0	38	25	23
9	50	7.0	4.0	5.0	<0.1	95	60	76
10	50	4.0	4.0	5.0	<0.1	44	80	31
11	50	4.0	40	0.3	<0.1	43	68	12
12	50	4.0	40	5.0	<0.1	44	69	17
13	50	4.0	40	0.3	5.0	38	37	24
14	50	7.0	4.0	0.3	5.0	70	38	85
15	50	4.0	4.0	0.3	5.0	42	39	12
16	50	4.0	4.0	0.3	<0.1	53	69	39
17	17	7.0	40	5.0	5.0	69	40	92
18	17	7.0	40	5.0	<0.1	89	79	69
19	17	7.0	40	0.3	5.0	70	40	82
20	17	7.0	4.0	5.0	5.0	68	45	93

21	17	4.0	40	5.0	5.0	20
22	17	7.0	40	0.3	<0.1	80
23	17	7.0	4.0	0.3	<0.1	76
24	17	4.0	4.0	5.0	5.0	36
25	17	7.0	4.0	5.0	<0.1	30
26	17	4.0	4.0	5.0	<0.1	78
27	17	4.0	40	0.3	<0.1	91
28	17	4.0	40	5.0	<0.1	79
29	17	4.0	40	0.3	5.0	27
30	17	7.0	4.0	0.3	5.0	22
31	17	4.0	4.0	5.0	5.0	22
32	17	4.0	4.0	0.3	47	22
33	33	5.5	22	2.5	0.5	22
34	33	5.5	22	2.5	0.5	23
35	33	5.5	22	2.5	0.5	24
36	33	5.5	22	2.5	0.5	90
37	33	5.5	22	2.5	0.5	42
						88
						59
						87
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microcosm study could be evaluated statistically by analysis of variance (ANOVA) using the slopes of the resulting straight-line plots.

Three transformations likely to linearize the results of the microcosm study are:

$$(\text{metal}) = \beta_1 / (\text{time}) + \beta_0 \quad (1)$$

$$\ln(\text{metal}) = \beta_1 \ln(\text{time}) + \beta_0 \quad (2)$$

$$\ln(\text{metal}) = \beta_1 (\text{time}) + \beta_0, \quad (3)$$

where (metal) is the aqueous metal concentration, time is the length of time after spiking of metal, and β_1 and β_0 are constants (Steele and Torrie 1960). Equation 3, which is the transformation for linearizing an exponential function of the type $M = \beta e^{-kt+b}$, was found to fit the data best, and therefore, each curve in the data set was transformed using equation 3. In all cases, the square of the correlation coefficient, r^2 , for the transformed plots was 0.85 or greater.

Once transformed, the slopes of each line were tabulated and compared using the F statistic from the ANOVA procedure in the statistical analysis system (SAS) program. The error term consisted of the five midpoint replicates and the higher order interaction values, since interaction beyond second order was not significant (at $\alpha = 0.01$). A complete discussion of the statistical methods employed here can be found in Youden (1951), Cochran and Cox (1957), and Mendenhall (1968).

The factors, levels, experimental design, and extent of metal removal from the microcosms are listed in Table VI-6. The percent removal of each metal is the percent change between initial and final metal concentrations and serves only as an approximate indicator of the rate of metal immobilization because the intervening data points were excluded from this calculation. The statistical analysis (ANOVA)

included all data points in the form of the slopes of the transformed curves. However, the physical meaning of the resulting slopes is less obvious than the percent removal, and therefore the latter were used to illustrate the treatment effects.

So that the microcosm results could be compared with the predicted equilibrium forms of each metal, the chemical components at the concentrations that existed in the water of the microcosms were evaluated by the computer program MINEQL (Morel and Morgan 1972; Westall et al. 1976). The MINEQL program is not flexible enough to allow case-specific ligands (e.g., humics) and their attendant constants to be incorporated in the equilibrium calculation, and therefore two ligands in the program, ethylenediamine (EN) and histidine (HIS), were selected as models of dissolved organic matter. These ligands were not intended to serve as molecular models of humic materials, since the small size and high nitrogen content of EN and HIS are not typical of humics. However, the conditional stability constants of EN and HIS with the heavy metals of interest are similar to those for the corresponding metal complexes with humic substances (Sillen and Martell 1971; Mantoura et al. 1978). For example, the average conditional constants reported by Mantoura et al. (1978) for aquatic humics with Cd, Cu, Mn, and Zn at pH 8 are $10^{4.6}$, $10^{8.8}$, $10^{3.4}$, and $10^{5.2}$, respectively. Corresponding values for HIS are $10^{4.7}$, $10^{9.7}$, $10^{2.7}$, and $10^{5.9}$, and for EN, the values are $10^{3.8}$, $10^{8.8}$, $10^{1.0}$, and $10^{4.0}$ for Cd, Cu, Mn, and Zn respectively. Hence EN and HIS were used to mimic the extent of metal binding by dissolved organic matter for the microcosm experiment.

1. Cadmium

During the 120-h experiment, cadmium in the microcosms was reduced from initial levels of approximately 10 µg/L to final concentrations ranging from a low of 0.4 µg/L to a high of 5.8 µg/L. These values correspond to 95% and 38% reductions of cadmium, respectively (Table VI-6). The experimental F statistics (obtained using ANOVA on the slopes of the transformed data) show that at the 99% confidence level, the factors pH and H₂S were correlated with aqueous cadmium concentrations (Table VI-7). In addition, the interaction between H₂S and pH was significant. Over the concentration ranges examined, DOC, iron, and calcium did not affect aqueous cadmium concentrations.

The level of pH strongly influenced the extent of cadmium immobilization, with 68–95% reductions occurring at pH 7, and 38–53% reductions at pH 4 (Table VI-6). Several mechanisms for decreasing aqueous metal concentrations are dependent on pH. For example, complexation of cadmium by organic matter, such as that in the peat sediments, is stronger at pH 7 than at pH 4 (Saar and Weber 1979). At low pH, hydrogen ion strongly competes with metals for the anionic functional groups such as carbonyl and phenolic moieties, whereas at high pH, the functional groups remain electron rich, which allows complexation of cations. In addition, the hydrolysis species of cadmium vary with pH, such that insoluble cadmium hydroxide species occur more readily at high pH than at low pH (Stumm and Morgan 1981). However, for the conditions that existed in the microcosms, the predicted form of cadmium is the simple aquated ion, Cd⁺² (except in the presence of H₂S) (Table VI-8). Thus, insoluble hydroxides of cadmium should not have been present at the pH levels used.

Table VI-7. Experimental F statistics for microcosm study.

	Cadmium	Copper	Manganese	Zinc
DOC	1.0	6.0	6.2	2.3
pH	<u>67.6*</u>	6.9	<u>96.6</u>	<u>150.9</u>
Ca	0.0	0.0	0.5	0.0
Fe	0.0	0.0	2.5	3.5
H ₂ S	<u>20.24</u>	<u>128</u>	6.8	<u>48.2</u>
DOC*pH	0.63	0.0	0.1	7.4
DOC*Ca	2.56	0.7	0.1	0.2
DOC*Fe	0.0	3.9	0.9	2.7
DOC*H ₂ S	4.67	<u>10.5</u>	2.4	0.3
pH*Ca	0.0	1.0	0.5	1.2
pH*Fe	3.97	5.9	0.0	0.1
pH*H ₂ S	<u>66.9</u>	7.8	4.8	<u>63.3</u>
Ca*Fe	0.44	0.0	2.0	0.6
Ca*H ₂ S	0.00	2.7	0.0	0.5
Fe*H ₂ S	1.5	0.1	0.34	1.5

*Underlined values significant at the 99% confidence level.

Table VI-8. Equilibrium forms of heavy metals predicted by MINERL program.

DOC	pH	H_2S	Predicted Forms			
			Cadmium	Copper	Manganese	Zinc
0	7	0	96% Cd^{+2} 4% $CdCl_1^+$	93% Cu^{+2} 7% $CuOH^+$	100% Mn^{+2}	100% Zn^{+2}
0	4	0	96% Cd^{+2} 4% $CdCl_1^+$	100% Cu^{+2}	100% Mn^{+2}	100% Zn^{+2}
10^{-5} M EN	7	0	96% Cd^{+2} 4% $CdCl_1^+$	89% $Cu(EN)_2$ 11% $CuEN$	100% Mn^{+2}	100% Zn^{+2}
10^{-5} M HIS	7	0	94% Cd^{+2} 3% $CdCl_1^+$ 3% $CdHIS$	88% $Cu(HIS)_2$ 12% $CuHIS$	100% Mn^{+2}	70% Zn^{+2} 30% $ZnHIS$
10^{-5} M EN	4	0	96% Cd^{+2} 4% $CdCl_1^+$	100% Cu^{+2}	100% Mn^{+2}	100% Zn^{+2}
10^{-5} M HIS	4	0	96% Cd^{+2} 4% $CdCl_1^+$	96% Cu^{+2} 4% $CuHIS$	100% Mn^{+2}	100% Zn^{+2}
10^{-5} M EN or HIS	7	5 mg/L	100% CdS	100% CuS	100% Mn^{+2}	100% ZnS
10^{-5} M EN or HIS	4	5 mg/L	100% CdS	100% CuS	100% Mn^{+2}	12% Zn^{+2} 88% ZnS

The other factor that controlled aqueous cadmium concentrations was H₂S, although contrary to expectations, the rate and extent of cadmium removal was less for the high H₂S levels than the corresponding treatments at low levels of H₂S. Apparently, the thermodynamically favored CdS precipitate (Table VI-8) was colloidal and remained in suspension during the time course of the experiment, since a fine precipitate was observed in the water (and also in the filtered water).

The results obtained for cadmium indicate that neither chemical precipitation as the sulfide or hydroxide of cadmium, nor adsorption or entrapment with hydrous oxides of iron is the primary mechanism of cadmium removal from water. Instead, it appears that complexation or adsorption by the organic peat sediment is the controlling mechanism of cadmium removal.

Additionally, the levels of spiked cadmium in the systems containing only surface water remained consistently high throughout the entire experiment (Table VI-9). These results support the conclusion that chemical precipitation and settling is not the initial mechanism of cadmium removal and that the organic peat was responsible for immobilizing cadmium from solution. However, the ultimate fate of cadmium may not be the organic peat, since after a period of time, the highly insoluble cadmium sulfide may become the predominant species.

2. Copper

Copper was spiked into each microcosm to a level of approximately 50 µg/L, and during the 120-h experiment, copper decreased to final concentrations ranging from a low of 7 µg/L to a high of 36 µg/L. The minimum reduction amounted to a 25% decrease, and the maximum reduction

Table VI-9. Reduction of metals added to surface water (no peat) during 120-h period.

DOC	pH	Conditions*			Percent Reduction**				Zinc
		Ca	Fe	H ₂ S	Cadmium	Copper	Manganese	Zinc	
50	7.0	4.0	5.0	<0.1	5	10	2	2	
17	7.0	4.0	5.0	<0.1	0	4	-3	3	
50	4.0	4.0	5.0	<0.1	3	6	0	8	
17	7.0	4.0	0.3	<0.1	8	0	0	0	
50	7.0	4.0	5.0	5.0	-3	2	6	3	
50	4.0	4.0	5.0	5.0	6	5	8	1	

*All values in mg/L except pH.

**Initial values: Cd, 10 µg/L; Cu, 50 µg/L; Mn, 100 µg/L; Zn, 100 µg/L.

corresponded to an 85% loss of aqueous copper (Table VI-6). The experimental F statistics indicate that for copper, the only significant main effect (at $\alpha = 0.01$) was from H_2S , although first-order interaction between DOC and H_2S was significant also. None of the other factors—DOC, pH, calcium, or iron—influenced the rate of copper loss from solution.

Hydrogen sulfide produced the same result for copper as for cadmium. In all cases, the rate and extent of metal loss was lowest for the highest level of H_2S (Table VI-6). Apparently the same phenomenon was occurring with copper as with cadmium, in that the predicted form of copper at an H_2S level of 5 mg/L was $CuS(s)$, which probably formed but remained suspended as colloidal material. These results suggest that if the metal sulfides form, large suspended particles that settle rapidly must be present (e.g., as a source of nucleation) if the sulfides are to assist in immobilizing metals in a swamp ecosystem.

Two of the factors that did not affect copper immobilization—pH and DOC—were expected, a priori, to alter the rate of copper loss. Copper complexation by dissolved organic matter is known to be pH-dependent, with stronger complexes at pH 7 than at pH 4 (Bresnahan et al. 1978; Buffle 1980). MINEQL computations using EN and HIS as model ligands for dissolved humic compounds show that the predicted forms of copper at pH 7 (in the absence of H_2S) are the organic complexes $[Cu(EN)_2; Cu(HIS)_2]$, whereas at pH 4, the predominant species is Cu^{+2} (Table VI-8). However, the strength of copper complexation by sediment-organic matter also increases with increasing pH (Bresnahan et al. 1978; Kerndorff and Schnitzer 1980). Perhaps a balancing effect

was occurring in the microcosms such that at pH 7, the dissolved organic matter was competing with the sediment-organic matter for copper, but at pH 4, despite the weaker copper-sediment interactions, little or no competition for copper existed from the dissolved organic matter (i.e., DOC). Thus, a similar rate of copper immobilization at both pH 4 and pH 7 could have been obtained.

Nevertheless, if the above process occurred, DOC should have controlled the rate of copper loss from solution. The lack of correlation between DOC and copper in the microcosm study could reflect the relatively high concentration of the lowest level of DOC (17 mg C/L). Possibly, dissolved organic matter was in excess even at the lowest concentration, and hence no concentration dependence was observed. Lower levels of DOC could not be tested because dissolution and release of organic matter from the sediments contributed significant amounts of DOC when levels were below 17 mg/L.

The results for copper indicate that adsorption to the peat sediment controls the aqueous levels of copper and that chemical precipitation is either a competing or secondary process. Data from the controls without sediment (Table VI-9) support the theory that adsorption to the sediments was the mechanism responsible for immobilizing copper, since, in the absence of sediments, the levels of copper remained within 10% of initial concentration.

3. Manganese

Aqueous manganese (II) levels in the microcosms declined from initial concentrations of 96–109 µg/L to final concentrations ranging

from a low of 8 µg/L to a high of 95 µg/L. The corresponding reductions ranged from 12% to 93% during the 120-h experiment (Table VI-6). The only factor that correlated significantly with loss of Mn(II) from solution was pH (Table VI-7). At pH 4, manganese decreased by 12–39%, but at pH 7 reductions of 69–93% were observed. Like the other metals, sorption to the sediments appears to have been the mechanism of Mn(II) removal. Furthermore, chemical precipitation and settling are unlikely to have caused the observed decreases since manganese levels remained high in the surface water systems devoid of sediments (Table VI-9). The lack of an observed correlation of manganese with H₂S or DOC was expected since, according to equilibrium calculations (MINEQL), Mn⁺² was the predominant form of manganese under all experimental conditions; thus precipitation as MnS or complexation with DOC should not have occurred.

4. Zinc

Zinc was added to the microcosms to an initial level of approximately 100 µg/L, and after 120 h the aqueous concentrations of zinc ranged from 20 to 86 µg/L, corresponding to reductions of 18–84% (Table VI-6). The experimental F statistics (Table VI-7) indicate that pH and H₂S were correlated with rates of zinc losses and that pH-H₂S interaction was significant. These results are similar to those obtained for cadmium, which is not surprising since the two metals are similar chemically (Cotton and Wilkinson 1966). For example, both cadmium and zinc are associated with naturally occurring organic matter at approximately equal strength that typically is intermediate between the strong copper-organic complexes and the weaker

manganese-organic complexes (Irving and Williams 1948; Mantoura et al. 1978; Kerndorff and Schnitzer 1980).

The most effective removals of zinc (67–84%) occurred at pH 7 in the absence of H₂S (Table VI-6). At pH 4 (and with <0.1 mg H₂S/L), zinc loss ranged from 22% to 33%. The addition of H₂S increased aqueous zinc levels in a manner similar to that for cadmium and copper; i.e., at pH 4 and 5 mg H₂S/L, zinc losses ranged from 15% to 28%, and at pH 7 and 5 mg H₂S/L, the range was 24–28%. The predicted species of zinc in the presence of H₂S (5.0 mg/L) was the insoluble ZnS (Table VI-8), which probably formed as a colloidal precipitate and remained in suspension.

Additionally, the surface water systems (without sediment) remained consistently high in zinc (Table VI-9), which further indicates that chemical precipitation as the hydroxide or sulfide was not the mechanism of zinc immobilization. It appears that for zinc, as well as for cadmium, copper, and manganese, adsorption to sediments was the mechanism of removal in the microcosms.

5. Summary

In summary, the parameters H₂S and pH were found to be the most important factors studied for the immobilization of metals in the model swamp ecosystems. The highest level of H₂S (5 mg/L) produced a slower rate of metal immobilization (except for manganese) than did the corresponding treatments at low H₂S levels (<0.1 mg/L). Apparently the formation of a colloidal precipitate that remained suspended caused these results. Variations in pH produced results that were similar for all four metals in that at pH 7, aqueous metal concentrations decreased more rapidly than at pH 4. Protonation of anionic functional groups, such as carboxyl and phenolic moieties in the organic peat, was the

likely cause of this effect. At pH 7, anionic functional groups, which complex metals, are more abundant than at pH 4 (Schnitzer and Khan 1972).

In no case did calcium affect aqueous metal concentrations in the microcosms. Bittell and Miller (1974) and Guy and Chakrabarti (1976) reported that calcium, at levels similar to those used here, effectively competed with metals for sites on insoluble organic and inorganic materials. Thus, based on their results, an increase in calcium in the microcosms was expected to decrease metal sorption to sediments. However, both Bittell and Miller (1974) and Guy and Chakrabarti (1976) used solid materials that contained much higher levels of heavy metals than did the sediment in this study. At the higher levels, Guy and Chakrabarti (1976) observed calcium replacement for only "weakly" bound metals; the weak sites complex metal ions only after the strong ones are filled. Perhaps at higher concentrations of metal ions or after a long period of metal addition to a swamp the "strong" sites would become saturated, and a calcium effect would be observed.

Iron did not influence immobilization of any of the four metals studied, which contradicts the results of others (Stumm et al. 1976; Davis and Leckie 1978; Vuceta and Morgan 1978). Under oxidizing conditions, the insoluble hydrous oxides of iron were expected to adsorb metals, which could either enhance the rate of metal removal for a rapidly settling floc or retard immobilization if the iron oxides were colloidal and remained suspended. Several reasons for this discrepancy are possible. In this study, iron oxides were formed in the presence of naturally occurring organic matter, and this could have influenced the nature of the resulting iron oxides. For example, Tipping (1981)

reported that humic substances adsorb to surfaces of hydrous iron oxides and alter the surface potential and sorption properties of the particulate iron.

Dissolved organic matter could have affected sorption of metals to iron oxides by another means, namely by complexing with metals and reducing their tendency to adsorb to solid surfaces (Jenne 1968; Rashid 1974; Guy et al. 1975; Davis and Leckie 1978). Of the four metals studied, copper is the most strongly adsorbed by iron oxides (Guy et al. 1975; Vuceta and Morgan 1978) and also the most strongly complexed with dissolved organic matter (Guy and Chakrabarti 1976; Mantoura et al. 1978). Cadmium, manganese, and zinc adsorb to iron oxides to a lesser extent than does copper, but their complexation with dissolved organic matter is also less. Hence, metal complexation by dissolved organic matter would decrease the extent of adsorption to iron oxides for all four metals studied, despite the variability in metal affinity of dissolved organic matter.

Additionally, the levels of iron and the other four metals investigated here were lower than the levels used in other studies (Stumm et al. 1976; Davis and Leckie 1978; Vuceta and Morgan 1978), and perhaps at the lower concentrations, sorption does not occur readily. Regardless of the reasons, it nevertheless is apparent that iron did not influence aqueous levels of cadmium, copper, manganese, or zinc under the conditions of this investigation.

Naturally occurring organic matter (i.e., DOC) did not influence the aqueous levels of cadmium, copper, manganese, or zinc over the concentration range examined (17–50 mg C/L). Others (Jenne 1968; Rashid 1974; Guy et al. 1975; Vuceta and Morgan 1978; Truitt and Weber 1979;

Sholkovitz and Copland 1981) have reported a marked effect on metal behavior in solutions with various complexing ligands, and a correlation of aqueous metal concentration with DOC was expected for this study. The organic matter from the water at Waldo Swamp was found to complex copper moderately strongly and the other metals to a lesser degree (see Chapters V and IV). Most likely, even at the lowest level of DOC tested (17 mg C/L), dissolved organic matter was in excess, and hence no correlation was observed. However, the presence of soluble organics undoubtedly influences the fate of metals in a swamp ecosystem.

For this study, the mechanism of immobilization of cadmium, copper, manganese, and zinc in the microcosms apparently was adsorption to the peat sediment. The ultimate fate of the four metals in sediments was not addressed here, but association with organic matter and precipitation as the highly insoluble sulfides during diagenesis are likely fates. For example, Nissenbaum and Swaine (1976) examined the forms of 18 metals in sediments and found that most of the copper and zinc was associated with sedimentary organic matter, whereas other metals such as nickel and cobalt were present as sulfides. In addition, Oakley et al. (1980) found that both insoluble organic and sulfide species were the dominant forms of cadmium, copper, and zinc in marine sediments. However, the rate and extent of formation of metal sulfides and other metal species during diagenesis remains in question.

CHAPTER VII CONCLUSIONS

The research reported in this dissertation consisted of 1) identifying the fate of heavy metals in freshwater wetland ecosystems, and 2) evaluating the interactions between heavy metals and soluble organic matter.

Monitoring efforts showed that the levels of Cd, Cu, Mn, Ni, Pb, and Zn entering Basin Swamp from the sewage treatment plant in Jasper, Florida, were low (0.1–30 µg/L). Consequently, no conclusions could be made regarding heavy metal cycling in Basin Swamp. The experiment conducted on a cypress swamp in Waldo, Florida, involved adding Cd, Cu, Mn, and Zn to septage from Waldo and pumping the wastewater through a 10 x 40-m corridor in the swamp. Soluble concentrations of all four metals decreased within the corridor to levels close to those considered acceptable for discharge to surface water. Rates of immobilization were calculated to be a minimum of 7.2, 36, and 72 and 72 g metal/ha·day⁻¹ for Cd, Cu, Mn, and Zn, respectively.

In addition to the field experiments, microcosms (model ecosystems that simulated a swamp) were constructed for a laboratory investigation of factors that control metal immobilization rates. Of the factors—dissolved organic carbon, iron, calcium, sulfide, and pH—the latter two most greatly affected metal immobilization rates.

The highest level of H₂S (5 mg/L) produced a slower rate of metal immobilization (except for manganese) than did the corresponding treatments at low H₂S levels (<0.1 mg/L). Apparently the formation of a colloidal precipitate that remained suspended caused these results. Variations in pH produced results that were similar for all four metals in that at pH 7, aqueous metal concentrations decreased more rapidly than at pH 4. Protonation of anionic functional groups, such as carboxyl and phenolic moieties in the organic peat, was the likely cause of this effect. The other parameters—calcium, iron, and dissolved organic carbon—did not influence immobilization of any of the four metals during the laboratory study. The primary mechanism of metal immobilization was adsorption to the peat sediment.

Five separate techniques were used to examine heavy metal interactions with naturally occurring organics and model compounds. Three of the techniques—anodic stripping voltammetry (ASV), ion-selective electrode, and fluorescence quenching—were previously published methods, and the other two methods—continuous flow ultrafiltration and competing ligand/differential spectroscopy—were developed for this study. Average copper binding capacities were $5.45 \cdot 10^{-5}$ M for Waldo Swamp water, $1.31 \cdot 10^{-5}$ M for Basin Swamp water, and $4.07 \cdot 10^{-4}$ M for a $3.7 \cdot 10^{-5}$ M polyaspartic acid solution. Copper was complexed moderately strongly with β' values ranging from $10^{4.87}$ to $10^{8.50}$. Binding capacities of cadmium and zinc were 5–10 times lower than those for copper. Values for both C_L and β' were similar among four the the five methods, with the ASV procedure yielding results that were considerably lower than the others.

Further evaluation of the ASV technique for speciating aqueous heavy metals showed that the method does not produce accurate results for copper. The ASV method yielded a conditional stability constant for copper with EDTA that was over five orders of magnitude lower than other published values. Work with naturally occurring organics showed that low ASV results were caused by the reducible nature of organically complexed copper.

Competitive binding studies using swamp-water organics and sewage organics indicated that, for the two swamps receiving sewage, the soluble form of copper entering the swamp would be controlled by the organics originally in the swamp water.

Experiments with peat-extractable organics showed that the peat was capable of binding 5.5 kg Cu/m³ of sediment due to the organic matter alone. The β' values indicate moderately stable complexation of copper by the peat extract. Consequently, the sediments in Waldo Swamp constitute a significant sink for copper and other heavy metals of similar or greater binding strengths.

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BIOGRAPHICAL SKETCH

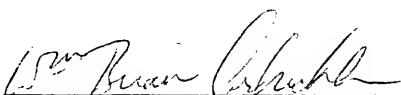
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He is married to Deborah Maday Tuschall.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


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This dissertation was submitted to the Graduate Faculty of the College of Engineering and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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